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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON NUMBERS OF SOIL MICROORGANISMS

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In a previous paper the author (11) gave a preliminary account of the general chemical and microbiological features of an English pasture soil under conditions of treatment which involved various combinations of manuring and irrigation. As a result of this work it was concluded that in grassland soils the upgrade and downgrade processes of the nitrogen cycle were no different in nature from those in arable soils, and that the observed differences in the levels of intensity of these factors were purely those which would be expected in view of the nature of the crop occupying the soil. It was concluded also that moisture exerted a controlling influence on numbers of bacteria, actinomyces, and fungi, in that numbers of these groups of organisms as indicated by plate counts were increased as a result of irrigation. At the same time, it was also suggested that the reduced numbers of these organisms which were recorded during the late autumn and early spring resulted from the lower soil temperatures then prevailing. The data, which consisted of weekly observations throughout the greater part of the year, have now been subjected to statistical treatment in an attempt to isolate the effects and to assess the relative importance of such environmental factors as soil moisture content, soil temperature, and soil reaction.

STATISTICAL TREATMENT OF THE DATA

The regressions of numbers of bacteria (b) and actinomyces (a) in millions/gm. dry soil, and fungi (f) in thousands/gm. dry soil, on moisture (m) as percentage of dry soil, temperature (t) in Fahrenheit degrees at 4-inch soil depth, and pH, during the 24 weeks period for which data were available, were investigated.

At the outset, some explanation is necessary concerning the particular method of analysis used. The figures, which exist for four differently treated plots (the four combinations of nitrogen, no nitrogen, irrigation, no irrigation), were formally analyzed as though they constituted a straightforward multiple

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regression problem. The inadequacy of this analysis, by itself, is considered in the discussion following the results. A much more complete analysis was in fact possible, for not only could the variation have been split up into positional (that is, treatment) and time effects, but the latter could have been divided further into long-term trends and short-term fluctuations. It should be pointed out, however, that if we are regarding the importance of different sources of variation in the number of organisms as proportional to the magnitude of the changes they produce, a major problem will be an explanation of the seasonal changes observed on the different plots. From this point of view, it is believed that the formal analysis made, provided it is associated with the discussion and the further statistical points made, has extracted practically all the information to be obtained from the data, and is a useful compromise in view of the very much greater computational labor attaching to any more complete analysis, and the limitations that, whatever the statistical treatment, this kind of data must inevitably have, if a consideration of trends is to be included.

TABLE 1

Variance extracted for different factors ($\times 10^4$)

	D.F.	*b*	*s*	*f*
m	1	26,971.1*	13,988.8*	183.9
t/*m*	1	111.9	14,790.5*	7,781.6*
tm/*t*, *m*	1	394.9	35.7	468.2
pH/*tm*, *t*, *m*	1	9.6	2,621.6†	719.8
Residual	91	330.3	452.0	319.5

* Significant, P = 0.01 (see below, however).

† Significant, P = 0.05 (see below, however).

Since it might be expected that variation in bacterial numbers would be directly proportional to the numbers themselves, the analysis was carried out on the logarithms of the numbers. The fluctuations observed justified this expectation and were made more comparable by this transformation. The fairly close relationship with moisture was also made more nearly linear. For consistency the logarithms of numbers of actinomyces and fungi were also used. In order to make sure that there was no evidence of an interaction between the effects of moisture and temperature on bacterial numbers, the variate *tm* was added.

Table 1 summarizes the results obtained in an analysis of variance form. The fraction of the variation in numbers of organisms that is expressible in terms of the "independent variates" *m*, *t*, *tm*, and *pH* has been extracted in stages corresponding to these variates in the order given. Thus the first row gives the variance corresponding to the simple regression on *m*; the second row, the extra variance accounted for in terms of *t* when this regression on *m* has already been eliminated, and so on. In this form the analysis of variance table is additive, and the total variance accounted for can be seen at a glance.

In table 2 are recorded the partial regression coefficients on *t*, *m*, and pH. Since from table 1 it can be seen that the inclusion of the variate *tm* has served no useful purpose, this has been omitted in preparing table 2.

The correlations between the "dependent variates" are given in table 3. The partial coefficients are those obtained after the elimination of the "independent variates."

It should be borne in mind that the foregoing regressions and correlations refer to the units in which the analysis was conducted, namely, *logs*.

Before any interpretation of the foregoing results can be attempted, some discussion is essential on the type of data that has been analyzed. In the first place, the dependence of bacterial numbers on external conditions such as moisture and temperature is a problem which theoretically could be most

TABLE 2

Partial regression coefficients on t, m, *and pH*

	b	*a*	*f*
t/*m*, pH	+0.00162	+0.01928*	+0.01390*
m/*t*, pH	+0.03560*	+0.05044*	+0.02118*
pH/*t*, *m*	−0.0107	−0.2634†	−0.1730

* Significant, P = 0.01 (see below, however).
† Significant, P = 0.05 (see below, however).

TABLE 3

Correlations between b, a, *and* f

	SIMPLE	PARTIAL
r_{ab}	+0.4673	+0.2778
r_{bf}	−0.0706	−0.2306
r_{af}	+0.1559	−0.1551

simply answered by a controlled experiment; but this is hardly possible in practice. (It should, however, be remembered that the irrigation of two of the plots modified the environment to the extent of producing a marked differential maintenance of moisture level during the driest spell.) The substitution of observational material must introduce limitations which will vary with any set of data available. The fluctuations will, for example, be different according as the data are similar observations at different places or successive observations at the same place. The data here correspond to the second class, except that four simultaneous observations for four plots exist for each week. There is no reason to suppose that these four observations, after any real effects of moisture, temperature, etc. have been eliminated, will be independent in a statistical sense, and furthermore there is no reason to suppose that the residual values of the successive observations from any one plot will be independent. Although in the statistical analysis these aspects have been formally ignored,

they have been borne in mind in assessing the validity of the conclusions which have been drawn. Finally, there is the important point already referred to, that since a fairly large fraction of the variation of the "independent variates" is fairly simple long-term change, the correspondence between, say, bacteria and one of these variates, even if statistically significant, from the viewpoint of regression, does not necessarily imply a real relationship. It is for this reason that the results have been stated in terms of regression and not of correlation. The terms given as significant are to be regarded only as meriting further examination, those not significant being assumed to be of no value. The dependent variates b, a, and f, especially after elimination of effects of environment, can be more legitimately regarded as chance variates, in connection with the correlations between them, but here again observations are not independent, so that, although it was of some interest to calculate correlation coefficients, no tests of significance were made. Any real correlations are somewhat reduced by errors in estimating b, a, and f.

It should be noted that since Jensen (16) also obtained his data under conditions approximating to the second class, namely, successive observations at the same place, the foregoing considerations are equally applicable, although he takes no account of this in making his deductions. For this reason doubt may attach to some of the correlations which he obtained.

With regard to the apparent association with environment, that of bacteria with moisture is greatest and has most claim to be real. Moreover, from a graph the relationship can be traced to some extent in the short-term fluctuations as well as in the trend, which, though markedly different for the irrigated and unirrigated plots, was closely followed in each case by the bacteria trend. Table 1 shows that nearly half the total variance is accounted for in terms of moisture and indicates also the absence of any other factor having any appreciable effect.

Since there appears to be least doubt about the influence of moisture in the case of bacteria, some idea of the magnitude of the effect of moisture on plate count numbers may be obtained with some justification from the partial regression coefficient given in table 1.

Since $\log b - \log b_0 = 0.0356\,(m - m_0)$,

$$b/b_0 = 10^{0.0356(m-m_0)}$$

$$= 1.085 \text{ (if } m - m_0 = 1)$$

That is to say, an increase of 1 per cent in the moisture content is accompanied by an increase of the order of 10 per cent in the numbers of bacteria. (This estimate is, of course, mainly determined by the seasonal variations in moisture content observed in the data.)

The strong relationship which exists between moisture and temperature naturally implies an apparent association between bacteria and temperature, but, with these data, there is no effect of temperature which is not fully accounted for in terms of moisture. The residuals for b, after elimination of m, have been plotted, and indicate further the dependence of the bacteria trend on that of moisture, constituting very fair approximations to random series.

The only exception is the series for the unirrigated N-treated plot; this gives a very slight but significant linear trend downward throughout the period, which may be ascribed perhaps to the nitrogen treatment.

It was expected that a relationship would be found with pH. In an attempt to ascertain whether any short-term relationship existed that had been masked by long-term changes, the first differences of the b residuals (m eliminated) were correlated with the corresponding pH difference series. No relationship was apparent, however, except for the control series. Here a coefficient of 0.556 was obtained, but this, in view of the selection involved and the lack of independence of the difference series, cannot be said to reach a significant level.

With regard to the fluctuations of actinomyces and fungi, neither show the same clear-cut relationship with moisture. The most prominent feature of the fungi series is, however, a tendency to rise throughout the period, and this bears a certain resemblance to the temperature (moisture eliminated) trend. The real significance of this trend, especially as no relationship with moisture was observed, must clearly remain a matter of doubt. It will be noticed that the partial regression coefficient of f on m appears significant, the slight resemblance of the fungi to the moisture series being brought out only when the trend effect mentioned above had been partly eliminated in terms of the variate t.

For actinomyces, the association appears equally divided between moisture and temperature. This again implies no more than the general similarity to the moisture or temperature trend, but the change in shape of the trend on the irrigated and unirrigated plots recalls the bacterial association with moisture and renders at least plausible the assumption of a real relationship of numbers of actinomyces with moisture. To investigate further the slight apparent association of a with pH, short-term fluctuations were examined by means of differences as for bacteria. The only apparent association was for the irrigated plots not receiving nitrogen, but even here r was only 0.336 and not significant. The slight relationship observed in table 1 has, therefore, been assumed to be arising spuriously from a trend effect, and has not been regarded as real.

With regard to the correlations between the dependent variates, it will be noticed that the negative correlation between bacteria and fungi is increased after elimination of the effects of moisture and temperature. This accords with Jensen's results (16). It will be noticed also that there is evidence of a simple positive correlation between bacteria and actinomyces, but this is reduced after elimination of temperature and moisture effects. Apart from the fact that Jensen also obtained a significant positive partial correlation between bacteria and actinomyces, the result receives some additional support from the fact that the simple and partial correlations are both positive, even though the method used to obtain the figures for b for dates subsequent to May 10 (that is, by subtracting from the total count the actinomyces count on *the same plates*) would tend to produce an inverse relationship.

Thus, to sum up, it is claimed that for the data examined, a clear-cut rela-

tionship exists between bacterial numbers, as shown by plate counts, and the moisture content of the soil. A similar, though somewhat less well-established, relationship appears to exist between the numbers of actinomyces and the moisture content, but a similar relationship in the case of fungi appears doubtful. For bacteria, no effect of temperature was observed which could not be fully accounted for in terms of moisture. For actinomyces and fungi the relationship is more obscure. There is no evidence that the pH of the soil, within the admittedly limited range experienced in this work (6.1 to 6.8), has exercised any effect on numbers of bacteria, actinomyces, or fungi. There is some slight evidence of a positive correlation between numbers of bacteria and actinomyces even after the effects of moisture and temperature (if any) have been eliminated, and in spite of the fact that errors in the method of obtaining the data tended to produce an opposite effect. Finally, there is some evidence of a negative correlation between bacteria and fungi.

DISCUSSION

The study of the causes underlying the variations in numbers of microorganisms has engaged the attention of numerous workers, but opinions have been by no means unanimous regarding any positive effects of moisture and temperature. There appears to be some measure of agreement, however, that temperature has relatively little effect. That organic matter is of overriding importance is, of course, clearly established, but in one and the same soil the change in the total amount of organic matter present during a season is of a much smaller order of magnitude than that which occurs between soils in different fields with different agricultural histories. Nevertheless, small changes in the amount or quality of a small fraction of the total organic matter may exert an important influence.

A review of the literature concerning the influence of moisture and temperature on numbers of soil organisms reveals the fact that little definite information has been obtained. Some conclusions that have been drawn do not bear careful scrutiny and under some circumstances have been unjustified and even invalid. Some observers have obviously ignored the fact that merely to demonstrate a correlation between two variables does not necessarily establish a causal relationship between them. Fabricius and von Feilitzen (13) claimed to show that the numbers of bacteria in a peat soil were influenced by temperature, but the paucity of the data leaves the matter open to doubt on statistical grounds. Engberding (12), working on soils on which various agricultural crops were growing, records a positive correlation between soil moisture and bacterial numbers, but it is doubtful whether the figures he gives reach a suitably high level of significance. He concluded that soil temperature had very little, if any, effect on numbers. Conn (4) records a correlation between total numbers of bacteria plus actinomyces and moisture in two soil plots, but his conclusion also is weakened by the fact that the data are not susceptible to statistical treatment. In 1916 Waksman (24) recorded his inability to show

any correlation between bacterial numbers and moisture or temperature; subsequently (25) he claimed to show a relationship between bacterial numbers and soil moisture content; the data as published, however, are too few to permit adequate statistical treatment. Crump made weekly counts in 1916–17, and found that bacterial numbers and moisture content varied directly much more frequently than they varied inversely. In 1922 Cutler, Crump, and Sandon (7), who were probably the first to realize the necessity for frequent and sustained observations, collected data every day for a year, but in spite of the abundance of their data, they were unable to satisfy themselves that any definite connection existed between the variations they observed in *fallow arable soil* and changes in such factors as temperature, moisture, or rainfall. They obtained, however, very convincing evidence of an inverse relationship, subsequently confirmed by Telegdy-Kováts (21), between numbers of bacteria and active amoebae. Referring to their work in a more recent publication (6), Cutler and Crump conclude that "the observed seasonal changes in numbers are due to an inherent urge in the spring and late autumn." No definite conclusions in respect of the effect of temperature and moisture can be drawn from the results of Smith and Worden (19), although the fluctuations which they observed were subsequently shown to be significant by Thornton and Fisher (22). Thornton and Gray (23) were also unable to trace any connection between bacterial numbers and moisture or temperature in arable soil, although they were able to demonstrate in one instance a parallelism between bacterial numbers and rainfall. In 1932 Cobb (3) recorded low numbers during drought, from which she inferred a causal relationship, but her data, which referred to forest soil, were far too few to permit adequate statistical treatment. Jensen (16) concludes that a "strong positive correlation existed between moisture content and numbers of bacteria, and a similar, though less well-pronounced, correlation between moisture and numbers of fungi, whereas the numbers of actinomyces did not seem to be influenced by changes in the moisture content." He goes on to say that "none of these groups of organisms showed any actual correlation with the temperature or any definite seasonal changes apart from those resulting from changes in moisture." His data, which consisted of about 50 observations collected at irregular intervals during 1931–1933 from the same pasture field in the grounds of Sydney University, were examined statistically by means of partial correlations.

As was shown in the foregoing section dealing with the statistical treatment of the data, very strong evidence of this positive correlation was also found, and there was a suggestion also that numbers of actinomyces and possibly also fungi were related in a similar way to the moisture status of the soil. Thus, as far as bacteria are concerned, the results here described are in agreement with those of Jensen, but contrast sharply with those of Cutler, Crump, and Sandon. It is submitted that the reason for this apparent contradiction is to be found in the fact that whereas the work of Cutler et al. was carried out in bare fallow soil, that is, in the *absence* of surface vegetation, both the work

here described and the work of Jensen were conducted in grassland soil, that is, in the *presence* of surface vegetation. At this point it is convenient to cite the results obtained by Wilson and Lyon, and by Starkey. Wilson and Lyon (27) showed that the numbers of various species of bacteria of such widely different physiological character as ammonifiers, nitrifiers, denitrifiers, and nitrogen fixers, when grown in previously sterilized soil, were very much greater in the presence of maize or timothy plants than in their absence. They concluded that the cause of the stimulation was the excretion of materials from the roots of the plants. Starkey (20) studied the effect of the stage of development of the plant on bacterial numbers, and found that for a large variety of plants, both in the greenhouse and in the field, the numbers of bacteria increased *pari passu* with the development of the plants, the maximum number being found at the period of maximum growth. He noted also that there was a rapid decline in numbers when the plants died. Numbers of actinomyces and filamentous fungi, however, showed little, if any, definite increase. In the light of these results there would appear to be little doubt that the numbers of bacteria in a pasture soil are influenced to a very large extent by the roots of the herbage, and that this influence will vary in proportion with the amount of herbage present. Furthermore, it is now only necessary to postulate that the supply of organic matter from the roots is closely associated with active growth of the herbage in order to arrive at a reasonable explanation of all the observed facts. It is suggested therefore that *the seasonal changes in temperature and moisture are not the direct cause of the seasonal changes in numbers of bacteria, but that in controlling the growth of the surface vegetation, these climatic factors control the amount of energy material reaching the bacteria as root excretions and also, in all probability, in the form of material sloughing away from the roots in the course of their development.* Presumably Cutler and co-workers and also Thornton and Gray found difficulty in correlating soil moisture content with changes in the level of numbers of bacteria because those changes in moisture content were unaccompanied by corresponding changes in the amount of energy material reaching the bacteria. In the experiments here described the increases in numbers of bacteria during irrigation were due, in all probability, not so much to the increase in moisture *qua* moisture, as to the increased supply of energy material reaching the bacteria as a consequence of the increased growth of the herbage which that moisture produced.

Evidence from other sources

Apart from the evidence already presented, further support is provided by the results of such work as has been done in other parts of the world under different climatic conditions from those obtaining in the British Isles. The only record of frequent and sustained observations conducted under tropical conditions is that given by Corbet (5), who made weekly counts of the numbers of bacteria and fungi in Malayan forest soils. The numbers he obtained were constant within narrow limits all the year round. The soil

moisture contents, under the conditions of his experience, were almost constant, and the soil temperature varied from 78 to 80°F. As might be anticipated from the considerations already outlined, he found no seasonal changes in numbers corresponding to those observed in temperate climates. The only detailed data of this nature forthcoming from the Southern Hemisphere, so far as the author is aware, are those already referred to, which were collected by Jensen from the pasture soil in New South Wales. On the basis of the suggestions put forward earlier, it may be inferred from the fact that the winters are considerably milder at Sydney, N. S. W., than anywhere in the British Isles, in consequence of which the grass barely stops growing during this time of the year, that the winter depression in numbers of bacteria would be much more restricted in duration and less pronounced, the tendency being thus to produce one winter peak. The figures given by Jensen certainly support this suggestion for the portion of the year which they cover. It should be noted (table 4) that 40 of his 50 counts were taken during the months August to December; consequently they cannot be expected to give information concerning the full range of any seasonal changes.

TABLE 4

Mean numbers of bacteria in grassland soils for various months [after Jensen (16)]

	WINTER			SPRING			SUMMER		
	June	July	August	September	October	November	December	January	February
Number of bacteria.....	.....	15	20	18.5	10.5	17.5	12.5	7.7	13.7
Number of observations....	.....	3	6	8	6	13	7	4	3

Similar considerations would lead one to expect that in some northerly latitudes, for example, in Finland, where grass production is more concentrated into the relatively shorter summer and where summer drought is more unusual and a longer winter is customary, the bacterial numbers would vary correspondingly, the two spring and autumn peaks which are experienced in England being resolved more or less into one summer peak. Unfortunately, no relevant data are forthcoming from Finland or any other country similarly placed with respect to climate.

Seasonal fluctuations in number of organisms other than bacteria

Numbers of many other types of soil organisms have been found to vary in a similar way according to the season. For example, Bodenheimer and Reich (2) have recorded seasonal fluctuations in numbers of protozoa in Palestine soils. Francé (14) claims that soil algae behave in a similar way. Periodicity of a like nature in the numbers of some fresh-water and marine organisms has been recorded from time to time (1, 8, 9, 15, 18, 26), and Dügelli (10) has shown that in Swiss pastures even the number of worms varies in the same way.

The soil food-chain

An exceedingly involved food-chain undoubtedly exists in the soil, and consequently it is most likely that a change in level of numbers of bacteria will bring about corresponding changes in the numbers of other organisms which may be vitally dependent on the bacteria directly or indirectly. The connection between bacteria and amoebae has already been mentioned, and by inference a whole host of similar relationships must exist, for it is only on such a basis that a state of biological equilibrium can be understood. It may be mentioned, parenthetically, that the inverse relationship between bacteria and amoebae, noted by Cutler and his co-workers, is a short-term relationship and not a long-term seasonal change. There is nothing inconsistent in the idea that a long-term direct relationship should be superimposed on a short-term inverse relationship.

Types of rhythmical behavior of organisms

The types of rhythmical behavior to which Cutler and Crump refer are not peculiar to microörganisms, but occur throughout the whole range of biology, and may be broadly classified as follows:

1. Changes occurring within an individual organism as a result of some force acting from within it.
2. Changes in a group of like organisms as a result of their action upon one another.
3. Changes in a group of organisms as a result of interaction with a rival or predatory species of organism.
4. Changes in the whole population of a species of organism resulting from changes in the incidence or intensity of purely external factors such as climate and food supply.

Although the existence of rhythmical behavior of organisms such as would be included in class 1 cannot be called into doubt, it would appear to be extremely unlikely that such phenomena would have any effect on the total numbers of organisms present, since there is no reason to expect the behavior of all the individuals to be in phase. If their behavior were observed to be in phase, it could be argued with considerable justification that their concerted behavior must result from the operation of some factor or factors external to the population as a whole, in which case the phenomenon could no longer be regarded as coming within the scope of class 1. The periodic ovulation of human beings, one of the examples quoted by Cutler and Crump, clearly falls into class 1, and on the basis of the foregoing argument can hardly be admitted as evidence of his contention. Furthermore, since it is only the type of rhythmical behavior covered by class 1 which can be most nearly described as "inherent" or "innate," it is submitted that the statements made by Cutler and Crump that "an inherent urge in the spring and the late autumn, . . . causes an increase in vital activity," and that "there is in protoplasm an innate capacity to behave rhythmically," contain no explanation and are no more than restatements of the problem.

There is, however, another type of rhythm which might be regarded as having some effect, namely, the phenomenon of impressed rhythm, where the organism continues to act rhythmically after the primary external factor causing the rhythm has ceased to operate. The well-known behavior of the two Turbellarian worms *Convoluta roscoffensis* and *C. paradoxa* is a case in point. These marine organisms adjust certain of their habits in a rhythmical way, which is in phase with the rise and fall of the tides, and Keeble (17) has shown that they continue to do so for a period of seven or eight tides when kept in the laboratory under suitable conditions of illumination. In the first place, this is a rhythm which is obviously due, under natural conditions, to the influence of an external factor, namely, the tides, however obscure the actual mechanism may be. The secondary effect, the impressed rhythm, is little understood, but in any case it seems in the highest degree improbable that rhythms of this description can be held to account for *seasonal* variations in bacterial numbers, since they are the property of each individual organism, and are not, so far as is known, transmitted through successive generations, as it would be necessary to postulate in the case of bacteria. Thus, it would appear that the long-term seasonal fluctuations in numbers of bacteria may be regarded almost certainly as having their origins in one or other of the factors listed in the three remaining classes. The observed fluctuations are unquestionably the resultant of several factors, some of which may be obscure or may operate indirectly; but however complicated the relationship may be, that they will be found in the last resort to be due to factors external to the organism, and not to causes resident within the living cell, appears equally certain.

The types of change covered by class 2 are usually of the logarithmic increase and decrease type, and refer to the behavior of a whole colony of like organisms. They arise essentially from local conditions of food and environment, the number of viable organisms increasing as conditions are favorable and decreasing with the exhaustion of vital necessities and the possible accumulation of staling products. Since a fluctuation in numbers composing one colony in one part of the soil cannot be linked to similar changes taking place in other colonies of similar organisms in other parts of the soil except through the operation of some external factor affecting all colonies alike, in which case the phenomenon should rightly come in class 4, the fluctuations in the various colonies must be regarded as being essentially out of phase. For this reason it is suggested that this type of fluctuation is also extremely unlikely to find expression as a seasonal variation in numbers.

The third type of fluctuation is that arising from the operation of the various factors inseparably connected with the state of dynamic equilibrium which exists between the various competing animal and vegetable soil forms. The inverse relationship which was demonstrated by Cutler, Crump, and Sandon to exist between active amoebae and bacteria, comes definitely within this class. Although in all likelihood it is one of the prime causes of short-term

variations, it would appear extremely doubtful whether the causes of the seasonal fluctuations can be assumed to arise in any way from such relationships. Even if the increase in bacterial numbers were to be associated with the periodic appearance of some relatively longer-lived organisms, such as worms, it is pertinent then to enquire why they should also appear at these times.

It is concluded that whereas short-term variations in numbers of microörganisms undoubtedly arise from what may be called competition factors, the long-term changes in numbers are the reflection of the seasonal changes in climatic conditions as affecting the supply of energy material to the microörganisms. Since this energy can become available to the heterotrophic organisms only via the medium of the green vegetation, their growth and activity should be closely associated with the growth and activity of the herbage. The available evidence certainly supports this view and suggests that the difficulty in tracing the seasonal changes in bare fallow soil is due to the absence of any marked increase in numbers of heterotrophic organisms at the appropriate seasons as a result of the bare fallow conditions, where the income of energy material to the soil system is restricted to that supplied by green algae or other small plants which may be able to survive bare fallow conditions.

SUMMARY

Weekly variations in numbers of bacteria, actinomyces, and fungi occurring in grassland soil have been examined in relation to changes in soil moisture content, soil temperature, and soil reaction. Statistical analysis has indicated a strong positive association between numbers of bacteria, as shown by plate counts, and the moisture status of the soil. Not only was this relationship apparent in untreated soil as a result of natural variations in soil moisture, but irrigation during the summer months also significantly increased the numbers of bacteria. A similar, though less well-established, relationship appeared to exist between the soil moisture content and the numbers of actinomyces and fungi. Between the limits experienced, soil temperature and soil reaction appeared to exert no measurable influence on numbers of organisms. It is suggested that the seasonal changes in moisture and temperature are not the direct causes of the seasonal changes in numbers of bacteria, but that in controlling the growth of the herbage, these climatic factors control the amount of energy material reaching the microörganisms in the form of root excretions or sloughed-off root material. Thus, in grassland soils the long-term changes in the numbers of microörganisms are closely associated with the amount and growth activity of the surface vegetation.

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THE EFFECT OF MOISTURE CHANGES ON SOIL AS A MEDIUM FOR BACTERIAL GROWTH[1]

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In a publication of 10 years ago (1) in which *Bacterium globiforme* was described, it was shown that two soils of low productivity would not support the growth of this organism unless furnished with available carbon and nitrogen sources. In the conclusion of this 1928 paper the following statement was made:

> The fact that these two soils lack all necessary carbon and nitrogen compounds to support the growth of *Bact. globiforme* is not improbably associated with their relatively low productivity. It is not suggested that this organism is necessary for the good growth of plants, but rather that general crops cannot thrive in a soil deficient in the substances required for the nutrition of *Bact. globiforme.*

Such a statement as this naturally suggested that the organism might be regarded as an indicator of productivity of a soil and gave rise to the understanding in numerous quarters that it was being thus used at the New York State Agricultural Experiment Station. As a matter of fact, no work with the organism has gone this far, as further investigations showed that the relationship between this organism and availability of soil nutrients was far from simple in nature.

Thus it was shown later (3) that the growth of the organism could be stimulated in some soils by the addition of a considerable variety of nonnitrogenous salts. It was concluded, "The only reasonable explanation seems to be that each of the salts having this stimulating effect has its influence because in some way it frees some nutrient element already in the soil but in unavailable form." If this were the case, the complex nature of the factors affecting the availability of the nutrients of this organism in soil became quite evident.

It was noticed, nevertheless, that stimulation of growth was most frequently observed following the addition of phosphates and potassium salts. It was felt, therefore, that these salts might serve directly as nutrients and that response of the organism to the addition of one of these salts in any particular soil might indicate deficiency of either potassium or phosphorus in that soil.

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[2] Acknowledgment is made to Jean E. Conn and Gladys E. Wolfe for assistance in obtaining many of the data in this paper.

Inasmuch as bacteriologists and mycologists have been trying for some time, but without entire success, to devise a quick test for soil deficiencies, it seemed worth while to investigate the possible use of *Bacterium globiforme* for this purpose.

During the investigation, a type of test was devised which has been used for the last 5 or 6 years upon a great variety of soils. Although it has not proved successful for its original purpose (detecting fertilizer deficiencies in soil), it has yielded interesting information as to the behavior of certain bacteria in soil.

METHODS

Details of the test are as follows:

Each soil employed in this work was carefully mixed before the test sample was taken. This was sometimes done after complete air drying of the soil, and at other times while the soil was still damp but not so wet as to puddle.

Four 10-gm. lots of the soil were weighed out, and each was mixed with certain ingredients. All lots of the soil were supplied with uniform quantities of glucose, as the source of carbon and energy, and of $CaCO_3$, to neutralize acids produced. In addition to this they were furnished K_2SO_4, K_2HPO_4, and $(NH_4)_2HPO_4$ in such combinations that one lot contained added N, P, and K; one lacked P; one lacked K; and one lacked both P and K. After preliminary experiments to determine the optimum amounts, the following quantities of these ingredients were added, respectively, to each of the four 10-gm. portions of the soil:

1. $(NH_4)_2HPO_4$, 0.03 gm. + $(NH_4)_2SO_4$, 0.03 gm. (lacking K); weight of NH_4:0.0164 gm.
2. $(NH_4)_2SO_4$, 0.05 gm. alone (lacking K and P); weight of NH_4:0.0136 gm.
3. $(NH_4)_2SO_4$, 0.05 gm. + K_2SO_4, 0.03 gm. (lacking P); weight of NH_4:0.0136 gm.
4. $(NH_4)_2HPO_4$, 0.03 gm. + K_2SO_4, 0.03 gm. (complete); weight of NH_4:0.0082 gm.

It will be noticed that none of the above combinations lack nitrogen. This is because preliminary work with soils furnished with only K_2SO_4 and K_2HPO_4 showed such uniformly low counts that they were of no value for comparative purposes. It will also be seen that the amount of N furnished (as NH_4) varies considerably. This is because the optimum quantity in the form of the phosphate proved, for some reason, to be smaller than that in the form of the sulfate.

After these ingredients were mixed with the soil, each portion was placed in a test tube. If the soil was air dry, 1 cc. of distilled water was added to it; but if the soil was moist, no water was added at this point. The tubes were plugged and sterilized in an autoclave for 1 hour at 15 pounds pressure.

The culture for inoculation had meanwhile been prepared by growing for 24 hours, at 30°C., on the surface of a slanted tube of beef-extract-peptone agar (in the case of *Bact. radiobacter*, this agar contained 0.2 per cent glucose). After the soil tubes were sterilized, the growth on the agar slant was rubbed up

in sterile water, and a definite amount of the suspension was transferred to the tube. If the amount of moisture in the soil *before* sterilization was 35 per cent of the water-holding capacity of the soil, 1 cc. of inoculum was added. If the soil was drier or wetter than this, the amount of inoculum was increased or decreased so as to make the final moisture conditions approximately uniform. (It was not found possible, unfortunately, to make the moisture conditions absolutely uniform, because the extent of the deflocculation due to the heating was so variable that the final water-holding capacity of the sterilized soil varied in an unpredictable way.)

The inoculated tubes were incubated 7 days at 30°C. (This temperature and the length of incubation were selected after considerable preliminary investigation to find how soon and under what conditions the bacteria attained their highest numbers.) The numbers of organisms in the soil were then counted by the microscopic method previously described (2). This method, in brief, is to suspend 0.5 gm. of soil in 4.5 cc. 0.015 per cent gelatin, to smear 0.01 cc. of the suspension over a measured area on a slide, and to stain with rose bengal solution (1 per cent in 5 per cent phenol with the addition of 0.01–0.02 per cent $CaCO_3$).

SEARCH FOR A SATISFACTORY TEST ORGANISM

As has been indicated, the use of *Bact. globiforme* did not prove a satisfactory indicator of nutrient deficiencies in the soil, although some of the early tests were promising. Investigations by the same method were, accordingly, begun with a number of other soil bacteria, mostly of the non-spore-forming, slow-liquefying group. No organism so promising as the first one investigated was discovered until work was undertaken with *Bact. radiobacter*, Löhnis.

The latter organism seemed at the beginning to show such striking responses to the influence of different soils and of the ingredients added to them that most of the remaining work was carried on with it. Table 1 is given here chiefly to show that these variations can be considerable and that there may be a certain degree of correlation between the counts attained by the organism and the productivity of the soil. The most striking points brought out in this table are as follows: The low counts with the first soil (which is not under cultivation); the very low counts of the second soil unless furnished either potassium or phosphorus; the striking response to phosphorus in the case of the third soil; and the very slight response to either potassium or phosphorus in the case of the last two soils. These four observations agree in a general way with the productivity of the soils, and fairly good agreement occurred upon repetition of the tests.

After this apparent correlation was found, some hundreds of determinations were made in hopes of demonstrating that the degree of response shown by this organism to such nutrient elements in several soils was proportional to the response shown by certain crops when grown in these same soils as indicated by greenhouse and field experiments. The hopes of using the tests in this way,

TABLE 1

Seven-day counts of Bacterium radiobacter *in soils supplied with glucose and $CaCO_3$ plus certain mineral salts; then sterilized and inoculated—moisture content before sterilization not controlled*

Figures indicate millions per 0.5 gm. of dry soil

	MINERAL SALTS ADDED*			
	$(NH_4)_2HPO_4$ $(NH_4)_2SO_4$ (lacking K)	$(NH_4)_2SO_4$ (lacking K and P)	$(NH_4)_2SO_4$ K_2SO_4 (lacking P)	$(NH_4)_2HPO_4$ K_2SO_4 (complete)
A marshy woodland soil	290	500	1,030	870†
A rather poor soil, especially deficient in K and P	1,120	390	1,230	1,380
A highly productive soil somewhat deficient in P	1,620	760	920	2,260
Two moderately productive soils responding chiefly to N fertilization	2,000	1,540	1,610	3,400
	1,770	1,540	1,740	2,780

* See "Methods."

† This count was made in soil given complete nutrients but in the form of $(NH_4)_2SO_4$ and K_2HPO_4.

TABLE 2

Counts of Bact. radiobacrr *in a single lot of air-dry soil (VS1), made by the same technic as that in table 1*

Figures indicate millions per 0.5 gm. of dry soil

DATE	MINERAL SALTS ADDED*			
	$(NH_4)_2HPO_4$ $(NH_4)_2SO_4$ (lacking K)	$(NH_4)_2SO_4$ (lacking K and P)	$(NH_4)_2SO_4$ K_2SO_4 (lacking P)	$(NH_4)_2HPO_4$ K_2SO_4 (complete)
May 1, 1934	2,030		2,070	1,670
Oct. 25, 1934		650		2,120
Jan. 7, 1935	990	265	375	1,195
Jan. 7, 1935	1,165	545	515	1,480
Feb. 4, 1935	615	205	350	1,205
Feb. 4, 1935	690	340	390	1,090
Feb. 4, 1935	900	240	535	1,025
Dec. 5, 1935	1,085	530	560	1,655
Dec. 5, 1935	695	430	560	1,375
Dec. 5, 1935	1,975	530	430	1,865
Dec. 5, 1935	1,180	385		1,580
June 2, 1937	1,350	860	1,120	1,755

* See "Methods."

however, did not prove to be justified. It was soon discovered that some factor or factors operated to cause irregularities in the numbers which the organism could attain in any soil.

Table 2 illustrates such a case. All the tests shown in this table were made with one soil of rather low productivity—Volusia silt loam from the Bare Hill

area west of Rushville, N. Y. It will be seen that the highest counts were obtained in the first two determinations. The data suggest that under optimum conditions this organism can attain a count of at least two billions per gram, provided the proper nutrients are added, but that in all but the first two tests some factor or factors prevented it from reaching these maximum numbers.

What could this factor be? The first suggestion, naturally, was that the culture had lost vigor when the later tests given in this table were made. Various methods of invigorating were tried, sometimes with moderate success and sometimes with none. It was finally concluded that too frequent transfers of a culture on artificial media decreased its ability to attain high numbers in soil, and that it was well, therefore, after every few months of work to go back to the stock culture and thus to obtain a strain that had not been so recently transferred on a fresh medium. Beyond this, no effort to invigorate the culture proved successful. Even under the best of conditions, there was plainly some factor other than the vigor of the culture which often operated to prevent its developing to maximum numbers.

EFFECT OF MOISTURE CHANGES

The first clue as to what this factor might be was obtained by noticing that soils in which it was impossible to obtain counts much over one billion were samples that had been kept for some time in air-dry condition. It began to be suspected that, in some way, keeping the soil in such a condition injured it as a medium for the bacteria under investigation. A preliminary experiment or two was planned, therefore, in which some of a soil giving low counts was kept moist for a few weeks before the tests were made. The results were interesting because when this moistened soil was mixed with the usual ingredients and inoculated, the counts obtained with complete nutrients were about twice as high as those that had been obtained when the air-dry soil was used. Accordingly, a more carefully controlled experiment was planned. In this experiment five soil samples were employed as follows:

VS1, the same lot of Volusia silt loam from Bare Hill employed in the earlier work (table 2).

H7 and H7b, two samples of Honeoye stony loam taken at different times from the same spot a few miles northwest of Phelps, N. Y.

P1, a sample of Plymouth sandy loam from near the shore of Long Island Sound, taken at Cold Spring Harbor, Long Island, N. Y.

VST1, a sample of Volusia stony silt loam taken from the Turkey Hill section, east of Ithaca, N. Y. Although similar in many respects to VS1, it showed much more tendency to puddle and to cake upon wetting.

The moisture-holding capacity of each soil was determined. Then four lots of each soil were prepared in duplicate—one kept air dry; the second moistened to 25 per cent of its water-holding capacity and kept in a tumbler covered with a Petri dish top; a third, similarly watered to 35 per cent; and a fourth, to 45 per cent of the water-holding capacity. These moistened soils were weighed

every few days, and the water that had been lost by evaporation was restored. At the end of 1 week, 2 weeks, and 3 weeks, respectively, 10-gm. samples of the various soils were mixed with glucose, $CaCO_3$, and the aforementioned combination No. 4 of mineral salts (containing N, P, and K). Each 10-gm. portion was placed in a test tube (1 cc. of water added to the air-dry soil). These tubes were then sterilized as usual; and one set was inoculated with *Bact. globiforme*, the other, with *Bact. radiobacter*, the bacterial growth being suspended in varying amounts of water so as to make the final moisture content of the different lots of soil comparable. The tubes were then incubated, and counts were made as usual on the seventh day.

TABLE 3

Seven-day counts in soils held at controlled moisture levels; then supplied with complete nutrients,† sterilized, and inoculated*

Figures indicate millions per 0.5 gm. of dry soil

SOIL‡	ORGANISM	AIR-DRY SOIL	HELD AT 25 PER CENT OF WATER-HOLDING CAPACITY, FOR			HELD AT 35 PER CENT OF WATER-HOLDING CAPACITY, FOR			HELD AT 45 PER CENT OF WATER-HOLDING CAPACITY, FOR			AVERAGE
			1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	
VS1	*radiobacter*	1,400	1,315	1,820	2,105	1,760	2,120	2,115	1,355	1,920	1,835	1,775
	globiforme	1,755	1,850	1,150	1,890	3,020	1,800	2,245	1,725	2,200	1,540	1,918
H7	*radiobacter*	1,335	1,715	1,740	1,515	1,665	2,090	2,125	1,345	2,280	1,955	1,777
	globiforme	1,050	1,735	1,225	1,620	2,170	1,400	1,685	1,750	1,670	2,040	1,635
H7b	*radiobacter*	1,278	2,090	2,360	2,178	2,068	2,478	2,260	2,113	2,495	2,318	2,164
	globiforme	1,160	1,795	2,028	2,183	2,253	2,503	2,365	2,100	2,103	2,530	2,102
P1	*radiobacter*	1,695	1,760	1,805	2,105	2,158	2,060	2,210	1,750	1,950	1,825	1,932
	globiforme	1,743	1,803	1,670	1,490	1,998	2,053	2,225	1,700	1,893	1,933	1,851
VST1	*radiobacter*	1,583	1,758	1,613	2,203	2,240	1,945	2,418	2,318	2,120	2,053	2,025
	globiforme	1,163	1,890	1,815	2,128	2,138	2,035	2,058	2,308	2,333	2,455	2,032
Treatment averages....		1,416	1,771	1,723	1,942	2,147	2,048	2,171	1,846	2,096	2,049	1,921
Aver. for	*radiobacter*	1,458	1,728	1,868	2,021	1,978	2,139	2,225	1,776	2,153	1,997	1,934
	globiforme	1,374	1,815	1,578	1,862	2,316	1,958	2,116	1,917	2,040	2,100	1,907

* The moisture treatment varied only *before* sterilization and reinoculation. The final moisture content of all the tubes was about 50 per cent of water-holding capacity.

† See "Methods."

‡ VS1, Volusia silt loam from Bare Hill; H7 and H7b, two samples of Honeoye stony loam; P1, a sample of Plymouth stony loam from Cold Spring Harbor; VST1, Volusia stony silt loam from Turkey Hill.

This experiment was too large to handle when all the tests were made simultaneously. The soil samples for this purpose, accordingly, were set up at

various times during a period of about 3 months. The results are summarized in table 3.

The work with soil VS1 was performed first and showed that this soil, with which high counts had not been obtained for some time, could be converted again into a good medium for the growth of the test organisms by proper moisture treatment preceding sterilization and reinoculation. The later tests with the other four samples tended to verify the results with VS1. The averages for the various moisture treatments, as given in the last three rows of the table, show a very marked improvement for the entire experiment when the soils were held at 35 per cent of their moisture-holding capacity for 1 to 3 weeks, almost as much improvement at the 45 per cent level, and some improve-

TABLE 4

Analysis of variance of counts given in table 3, from air-dry soil and the soils held for 3 weeks at varying moisture levels

SOURCE OF VARIATION		DEGREES OF FREEDOM	SUM OF SQUARES OF DEVIATIONS*	MEAN SQUARES*	RATIO OF MEAN SQUARE TO ERROR (F)	COMMENT
Total		39	6.2993			
Between means of	soils	4	0.6851	0.1713	2.21	To be significant F should be greater than 3.26
	organisms	1	0.0391	0.0391	0.503	To be significant F should be greater than 4.75
	moisture levels	3	3.3129	1.1043	14.2	Any value of F over 5.95 is *highly* significant
Interactions:						
soil-organisms		4	0.0501	0.0125		
moisture-organisms		3	0.0997	0.0332		
moisture-soils		12	1.1627	0.0969		
Remainder (error)		12	0.9587	0.0779		

* The counts given in table 3 were converted to billions (decimal point moved 3 places farther to the left) before these computations were made.

ment at the 25 per cent level. The improvement apparently took place slowly at the 25 per cent level, continuing, in fact, for the whole 3 weeks of the experiment; whereas at the 35 per cent level there was no further improvement after the end of the first week.

It is also noticeable from table 3 that two of the soil samples, namely VST1 and H7b, showed almost uniformly higher counts than did any of the other samples. Brief inspection of the table does not show, however, whether these differences were of any significance. It is striking that the highest and the lowest averages were obtained, respectively, with H7b and H7, the two samples obtained from exactly the same spot in the field; this certainly suggests that the differences between soils are not significant.

Another point of interest is that the final averages for *Bacterium radiobacter* and *Bacterium globiforme*, respectively, were almost exactly the same. This observation, together with the fact that low counts of one organism usually paired with low counts of the other, and *vice versa*, suggests that both of these organisms behave about the same in the soils studied and under the conditions of this experiment.

Although these conclusions seemed fairly reliable, it was not regarded as safe to trust them without statistical analysis of the data. These were, accordingly, analyzed for variance by the method outlined by Snedecor (4). The results are given in table 4. In this table the values of F given in the fifth column are striking. The value of F for the soils is almost, but not quite, high enough to be significant; that for the organisms is not only too small to be significant but so low that it can be virtually disregarded; whereas that for the moisture levels ($F=14.2$) is more than twice as high as the value 5.95, which would be *highly* significant. From these values it was reasoned that the conclusions given in the preceding paragraphs are justified, that is, the moisture level of the soil before sterilization and reinoculation has a very decided effect on results, the differences between the soils are not great enough to be significant, and the two organisms employed seem to behave in exactly the same manner.

The similarity between the results obtained with the two organisms is regarded as very important. It seems to indicate reliability of this method of comparing soil treatments. Although not all the differences observed can yet be explained, it is felt that they are real differences, and that present inability to interpret some of them is due to lack of understanding of all the factors involved.

A POSSIBLE EXPLANATION

The effects of varying moisture levels in the soils were a little surprising and seemed at first somewhat difficult to explain. If the final moisture content of the soil in the culture tubes had varied, the explanation would be simple; but since it was kept as constant as possible, the explanation evidently must be more complex. Apparently in some way a soil that has been kept in air-dry condition may become a less favorable medium for the growth of these organisms than the same soil that has been kept moist for 2 or 3 weeks before sterilization and addition of the nutrients necessary to support the bacteria. Only two possible explanations have occurred to the writers:

1. When a soil is kept moist for 2 or 3 weeks, the bacteria therein must, obviously, be more active than when it is in an air-dry condition. Possibly they produce growth-accessory substances or in other ways render the soil more suitable for the bacteria subsequently inoculated into it than when kept in an air-dry condition.

2. Volk (5) has shown that alternate wetting and drying of soils treated with soluble potassium salts causes rapid fixation of the potassium in a nonreplaceable form. It is entirely possible, therefore, that some such phenomenon might occur under the conditions of this experiment. Keeping the soil air-dry for a considerable period may fix the potash, or some

other necessary base present in the soil, to such an extent that the regular quantities of nutrients added to the culture tubes in this experiment were insufficient to permit maximum growth of the bacteria. It is, of course, also possible that the condition of the colloids in the soil after standing for some time in an air-dry condition might be such that not only the potassium already present in the soil, but that added to the culture tubes in the experiment, might be fixed.

The data reported in table 5 were obtained in the course of a series of experiments to determine which of these two explanations seemed the more probable. In these experiments, three soil treatments, as follows, were compared: One consisted of keeping the soil unsterilized and air-dry; the second, of bringing it up to 35 per cent of its water-holding capacity and maintaining it at this moisture level without sterilization for 3 weeks; the third, of adding to it the same amount of water as in the second treatment, but of sterilizing it in the autoclave and keeping it in cotton-stoppered flasks. Soil given the third treatment was divided into two portions, each in a separate flask, one of which was opened after 2 weeks, the second after three weeks; this was to prevent contamination of the portion tested the third week when the 2-week test was made.

Two soil samples were employed in this series of experiments, sample VS1 in the first seven experiments, and sample VST1 in the last three. After the samples had been kept moistened for the requisite length of time, they were mixed with the usual amounts of glucose and calcium carbonate and with varying quantities of K_2SO_4 and $(NH_4)_2HPO_4$. In the third and fourth columns of table 5 the quantities of these last two chemicals are given, the figures in bold-face type indicating the quantities that were above the standard amounts employed in the preceding work.

In planning this experiment it was realized, of course, that sterilization not only would prevent bacterial growth but might produce physical and chemical changes in the soil and consequently that it would not prove a perfect control on the effect of bacterial activities. Whether from this cause or some other factor, these experiments did, as a matter of fact, prove inconclusive. The counts obtained in the soil that had been moistened and then sterilized were usually higher than those in the soil that had been kept air-dry, but they were rarely as high as those in the soil which had been moistened but not sterilized; they did not come so near to equalling the latter counts in soil VST1 as they did in soil VS1. This suggests that part of the explanation of the higher counts in the moistened soil may be the effect of the bacteria that grow in it during the period when it is kept moist, but because there was no way of separating the bacterial effects from the physical and chemical action of the heat, this conclusion is not well established.

Those tests which were planned to show whether fixation of some base occurred in the air-dry soil were interesting, but likewise inconclusive. In soil VS1 distinctly higher counts were obtained when the quantities of potassium sulfate were increased slightly above the standard amount employed in the previous experiments. In some instances the counts obtained with twice the

standard quantity of this salt almost equalled those obtained in the unsterilized moistened soil. With soil VST1, the same tendency was observed, but the counts did not come so near to equalling those obtained in the moistened soil.

TABLE 5

Seven-day counts in soils held at controlled moisture levels, either sterilized or unsterilized, then supplied with varying quantities of salts before inoculation

Count figures indicate millions per 0.5 gm. of dry soil

EXPERIMENT NUMBER	ORGANISM	NUTRIENTS ADDED TO 10 GM. SOIL (IN ADDITION TO 0.1 GM. GLUCOSE AND 1 GM. $CaCO_3$)		COUNTS IN UNSTERILIZED AIR-DRY SOIL		COUNTS IN UNSTERILIZED SOIL HELD AT 35 PER CENT OF WATER-HOLDING CAPACITY, FOR		COUNTS IN STERILIZED SOIL HELD AT 35 PER CENT OF WATER-HOLDING CAPACITY, FOR	
		K_2SO_4	$(NH_4)_2HPO_4$	First test	Second test	2 weeks	3 weeks	2 weeks	3 weeks
		gm.	gm.						
				Soil VS1					
1	*Radiobacter*	0.03	0.03	860	955	1,900	1,820	1,285	1,130
	Globiforme	0.03	0.03	635	1,500	1,310	1,500	1,250	2,027
2	*Radiobacter*	0.03	0.03	500	1,095	1,610	2,240	905	935
	Globiforme	0.03	0.03	960	895	1,830	1,610	790	1,415
3	*Radiobacter*	0.03	0.03	1,420	1,385	1,950	2,065		
	Globiforme	0.03	0.03	970	840	1,435	2,620		
4	*Globiforme*	0.03	0.03	625	1,175	2,215	1,635		
	"	**0.06**	0.03	880	1,220	1,575	1,340		
	"	**0.1**	0.03	1,130	1,425	1,670	1,385		
	"	0.03	**0.06**	1,100	800	1,465	1,245		
	"	0.03	**0.1**	700	720	750	1,390		
5	*Globiforme*	0.03	0.03	935	990	2,045	1,990		
	"	**0.06**	0.03	1,120	1,225	2,305	1,660		
	"	**0.1**	0.03	1,635	1,320	1,450	1,810		
	"	**0.15**	0.03	1,515	1,065	1,215	1,160		
	"	**0.20**	0.03	1,065	1,005	1,135	1,690		
	"	0.03	**0.06**	1,685	1,570	1,245	1,385		
	"	0.03	**0.1**	1,335	1,360	1,530	1,790		
6	*Globiforme*	0.03	0.03	1,090	1,105	2,510	1,980	1,585	1,910
	"	**0.06**	0.03	1,710	2,005			2,060	1,255
	"	**0.1**	0.03	1,240	1,285			1,990	950
	"	**0.15**	0.03	1,305	1,190			1,680	755
7	*Globiforme*	0.03	0.03	1,260	1,235	2,415	2,120	1,475	1,480
	"	**0.06**	0.03	1,810	1,680			1,875	1,710
	"	**0.1**	0.03	1,545	1,510			2,235	1,240
	"	**0.15**	0.03	1,305	1,360			1,575	1,100

TABLE 5—*Concluded*

EXPERIMENT NUMBER	ORGANISM	NUTRIENTS ADDED TO 10 GM. SOIL (IN ADDITION TO 0.1 GM. GLUCOSE AND 1 GM. $CaCO_3$)		COUNTS IN UNSTERILIZED AIR-DRY SOIL		COUNTS IN UNSTERILIZED SOIL HELD AT 35 PER CENT OF WATER-HOLDING CAPACITY, FOR		COUNTS IN STERILIZED SOIL HELD AT 35 PER CENT OF WATER-HOLDING CAPACITY, FOR	
		K_2SO_4	$(NH_4)_2HPO_4$	First test	Second test	2 weeks	3 weeks	2 weeks	3 weeks
		gm.	*gm.*						
				Soil VST1					
8	*Radiobacter*	0.03	0.03	970	1,080	2,140	1,685	1,095	1,220
	"	**0.06**	0.03	1,170	1,165	1,930	1,675	1,770	1,175
	"	**0.10**	0.03	1,225	1,560	1,925	1,715	1,250	1,050
	"	**0.06**	**0.06**	1,170	995	1,775	1,220	1,070	935
	"	0.03	**0.06**	1,220	1,285	1,800	1,545	1,245	1,060
	"	0.03	**0.10**	1,240	1,200	1,760	1,315	1,190	1,060
9	*Radiobacter*	0.03	0.03	795	655	1,790	1,070	1,070	910
	"	**0.06**	0.03	1,050	965	2,145	1,200	955	980
	"	**0.10**	0.03	1,085	1,230	1,595	1,650	890	780
	"	**0.06**	**0.06**	900	780	1,340	1,080	835	785
	"	0.03	**0.06**	1,040	945	1,720	1,300	825	870
	"	0.03	**0.10**	1,060	860	1,585	1,185	820	970
10	*Radiobacter*	0.03	0.03	815	1,320	1,070	2,110	670	1,210
	"	**0.06**	0.03	810	1,640	955	2,015	720	1,320
	"	**0.10**	0.03	1,085	1,655	890	2,020	800	1,150
	"	**0.06**	**0.06**	825	1,455	835	1,990	505	960
	"	0.03	**0.06**	945	1,665	825	2,005	675	1,150
	"	0.03	**0.10**	805	1,490	820	1,990	520	1,030

Increasing the amount of ammonium phosphate, however, proved as apt to decrease as to increase the counts.

This would seem to indicate that the addition of increasing amounts of potassium sulfate to the air-dry soil improves it, at least up to a certain point, for the growth of bacteria. In the moistened soil, however, the effect was quite different. The same quantities of additional potassium sulfate which seemed to improve the air-dry soil, were very apt to have a depressing effect on the count in the moistened soil. The results, however, were too irregular to allow any very definite conclusion.

They suggest, nevertheless, that there is a delicate balance of nutrients in soil which may easily be upset by some change in the colloidal complex, fixation of potassium possibly being one factor involved. Apparently the quantities of potassium sulfate and ammonium phosphate which were employed regularly in the experiments reported in table 3 were optimum, ordinarily, for the bacteria in question when these salts were added to soil that had been kept at 35 per cent of its moisture-holding capacity for 2 or possibly 3 weeks. Under

such conditions increasing the quantity of salts added usually decreases the count obtained. If, however, because of drying of the soil, sterilization, or too long holding in a moist condition, considerable of the potassium, or possibly some other base, becomes fixed, the amounts of salts regularly added in this work are insufficient to produce maximum counts; and increases in numbers of the bacteria can, therefore, be obtained by increasing the quantities added. Most noticeable results were obtained in these experiments by increasing the quantities of potassium sulfate. This suggests that potassium is the critical element; but it does not prove the case, because the phenomenon of base exchange may take place, and consequently adding this salt might increase the availability of some other cation.

Whatever the explanation, it is very clear from these later experiments, as well as from those reported in table 2, that the soil *is* a better medium for the growth of these bacteria if it has been kept moist for a period of about 2 weeks. Such results go far to explain the difficulties encountered in trying to use this type of bacteriological procedure as a test for nutrient deficiency in soil. It is difficult to compare plant nutrition with that of microorganisms. Plants live through many changes in weather and accordingly in the moisture level of the soil in which they are growing. If, as the present results seem to indicate, such changes impair or improve the soil as a place for growth, possibly by fixing or liberating necessary nutrient elements, the consequences are naturally less serious to higher plants than to microorganisms which have a short life period and are accordingly more strongly affected by the conditions which obtain during that brief period. This may well explain one of the factors which underlies the difficulties encountered by soil biologists who have been trying to devise tests for this purpose, whether the test organism be a bacterium, a yeast, or a mold. It is certain, at least, that in the present instance the great fluctuations in counts obtained when the nutrients supplied were not varied indicate the operation of some factor which makes such a procedure of dubious value as a test for nutrient deficiencies.

SUMMARY

The experiments reported in this paper were begun in an effort to study nutrient deficiencies of soils by the use of either *Bacterium radiobacter* or *Bact. globiforme* as the test organism. Growth of the test organism in tubes of sterilized soil, with varying nutrient elements added, was determined by a microscopic count 7 days after inoculation.

Although the method has not yet proved useful for the purpose originally intended, it did bring out certain points of considerable interest; to wit:

Almost identical results were obtained with the two test organisms whenever they were directly compared. This seemed to indicate that the test is dependable, and that the differences it brings out are real differences, even though it is not yet possible in every case to explain them on the basis of treatment or soil conditions.

Minor differences were observed between the various soils studied, but statistical analysis proved these differences too small to be significant.

The soil samples studied (five in number), if allowed to become air dry, were impaired as media for growth of the test organisms, even after they were brought up to optimum moisture content and the necessary nutrients added to support bacterial growth.

It proved possible to restore air-dry soil to its normal condition in this respect by holding it at 35 per cent of its water-holding capacity for about 2 weeks before adding the bacterial nutrients, sterilizing, and inoculating with the test organisms.

Experiments were carried on to show whether the improvement of the soil after moistening might be due to changes brought about in it by growth of the natural soil flora during the 2-week period while the soil was moist, or by release during this period of bases that might possibly have been fixed by the soil colloids while the soil was in an air-dry condition. Indications were obtained that fixation of potassium might be one of the factors involved; but results varied too greatly to be conclusive.

Changes in the condition of the nutrients due to variations in the moisture level may well be more serious to bacteria, studied for a week or so after inoculation, than to plants which live months, usually through many changes in moisture conditions. This may explain one of the difficulties encountered in using microorganisms to indicate plant nutrient deficiencies.

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THE CHEMICAL COMPOSITION OF SOIL FROM CULTIVATED LAND AND FROM LAND ABANDONED TO GRASS AND WEEDS[1]

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Reference has been made in previous publications (2, 5, 6) to the fact that it is when land goes under the plow that deterioration begins. The unbroken prairie or land abandoned to natural grasses and weeds may not change very much in chemical composition over a long period of years. There is, however, a fair probability that it may gain slightly, especially in content of nitrogen and organic matter.

When the soil fertility plots were laid out at the New Jersey Agricultural Experiment Station in 1908, provision was made for eight 1/20-acre plots, one-half of each plot to be continuously in a 5-year rotation (corn, oats, wheat, and 2 years of timothy), the other half to be abandoned to grass and weeds (2). The fertilizer and lime treatment for these plots were to be the same for both sections. The plan has been adhered to to date with only slight exception. By 1923, dewberry vines were overrunning some of the plots of the uncultivated section, and for this reason it seemed best to plow them under. Corn was grown on all the plots of this section for the one season only. No other crops have been removed. Some years the growth of weeds and grasses has been allowed to stand; more often it has been clipped with the mowing machine and allowed to lie on the ground, the next year's growth coming up through the dead material.

The fertilizer combinations used on these plots were as follows:

Plot number	*Fertilizers*
57 and 58	P and K
59 and 60	N and K
61 and 62	N and P
63 and 64	N, P, and K

Originally, the nitrogen was supplied in the form of ground fish at the rate of 600 pounds per acre. Later, the nitrogen was supplied in the form of a mixture of nitrate of soda and sulfate of ammonia to give a total of 51 pounds of nitrogen per acre (half the nitrogen from nitrate of soda and half from sul-

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fate of ammonia). Superphosphate was originally applied at the rate of 600 pounds per acre; and muriate of potash, at the rate of 300 pounds per acre. These amounts were later reduced to 300 pounds and 150 pounds respectively. Lime in the carbonate form has been added from time to time to maintain the pH at about 6.0–6.5.

The purpose of this paper is to show the changes that have occurred in the chemical composition of these soils over a period of 30 years, with especial reference to certain total constituents and changes in certain physicochemical properties.

EXPERIMENTAL

In July 1937, soil samples were collected from these plots for chemical analysis. The surface samples were taken to a depth of $6\frac{2}{3}$ inches and the subsurface samples from $6\frac{2}{3}$–12 inches. In addition four plots from the uncultivated section were sampled at successive 2-inch depths down to 6 inches.

The total nitrogen, phosphoric acid, and potash were determined by A. O. A. C. methods (1). The carbon content of the 1937 samples was determined by the wet combustion method of Tiurin (11). The cation exchange capacity was determined by the barium acetate method (8). The exchangeable calcium, magnesium, and potassium were displaced from the soils either by the normal ammonium acetate method or by electrodialysis (9), and these cations were determined in the extracts by the usual methods (1). Exchangeable hydrogen was calculated by subtracting the sum of the exchangeable calcium, magnesium, and potash from the total cation exchange capacity.

The pH of samples as taken (field pH) and ultimate pH values were determined by the glass electrode on 1–1 soil suspensions.

CHANGES IN NITROGEN AND CARBON CONTENT OF CULTIVATED AND UNCULTIVATED SOILS OVER A 30-YEAR PERIOD

Table 1 gives the results of nitrogen and carbon determinations made on samples from the cultivated and uncultivated sections of plots 57 to 64 in 1913, 1916, and 1937. This table brings out the fact that, without exception, both nitrogen and carbon are now higher in the uncultivated section than in the cultivated.

The average difference between the two sections in nitrogen content of the nitrogen-treated plots, was 0.02 per cent in 1913, 0.023 per cent in 1916, and 0.067 per cent in 1937. In pounds of nitrogen per acre $6\frac{2}{3}$ inches (2,000,000 pounds of soil), these values would correspond to 400, 460, and 1,340 pounds respectively. The average nitrogen difference between the cultivated and the uncultivated sections of the plots that receive no nitrogen was 0.012 per cent in 1913, 0.026 per cent in 1916, and 0.039 per cent in 1937. These values correspond to 240, 520, and 780 pounds of nitrogen to the acre respectively.

The results are in accord with previously published data (3, 4) showing that liberal applications of phosphorus, potassium, and calcium favor the appear-

ance of volunteer leguminous plants on abandoned land and also the fixation of atmospheric nitrogen by nonsymbiotic bacteria, consequences which no doubt account for the fact that on the uncultivated sections of these plots the nitrogen supply is fairly well maintained. Volunteer leguminous plants, especially clovers, also appeared in the no-nitrogen plots of the cultivated section following timothy or grain, but their presence did not appreciably influence the nitrogen content of the soil. Scarcely any volunteer legumes appeared on the nitrogen-treated plots of the cultivated section.

On the basis of an average of all the nitrogen-treated plots, the cultivated section shows slightly less nitrogen in 1916 than in 1913 and still less in 1937, in spite of the fact that the plots have received fair annual applications of nitrogenous fertilizers. For the uncultivated section, the average is the same in 1913 and 1916, but a gain of 0.039 per cent by 1937 is noted.

TABLE 1

Nitrogen and carbon in soil, 1913, 1916, and 1937

TREATMENT	CULTIVATED SECTION						UNCULTIVATED SECTION					
	Nitrogen			Carbon			Nitrogen			Carbon		
	1913	1916	1937	1913	1916	1937	1913	1916	1937	1913	1916	1937
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P and K	0.085	0.073	0.077	1.012	0.999	0.975	0.097	0.099	0.116	1.225	1.272	1.529
N and K	0.086	0.081	0.081	1.095	1.067	0.967	0.099	0.102	0.155	1.272	1.306	1.906
N and P	0.084	0.081	0.070	0.997	0.979	0.863	0.107	0.106	0.146	1.352	1.382	1.848
N, P, and K	0.084	0.083	0.080	1.035	1.062	0.975	0.108	0.107	0.132	1.323	1.334	1.696
Average of N-treated plots	0.085	0.082	0.077	1.042	1.036	0.935	0.105	0.105	0.144	1.316	1.341	1.817

It is evident that the total carbon is being gradually decreased on the cultivated section. This is shown by an average percentage of 1.042 in 1913, 1.036 in 1916, and 0.935 in 1937. It is further emphasized by the fact that determinations made on samples collected in 1909 from nearby plots, where the soil is the same as the soil of the plots under consideration, show 1.21 per cent of total carbon (2). From this it would appear that the cultivated plots have lost 0.17 per cent during the period from 1909 to 1916 and 0.275 per cent during the period from 1909 to 1937. On the other hand, the carbon is increasing in the uncultivated plots as is shown by an average of 1.316 per cent in 1913, 1.341 per cent in 1916, and 1.817 per cent in 1937. Thus, it would appear that during the period 1909 to 1916 the carbon content of the uncultivated soils has increased by about 0.13 per cent, which would be equivalent to slightly more than $1\frac{1}{4}$ tons of carbon per acre. During the period from 1909 to 1937 the carbon content has increased by 0.607 per cent, which would be equivalent to about 6 tons of carbon per acre. The gains and losses in nitrogen and carbon content are shown graphically in figures 1 and 2.

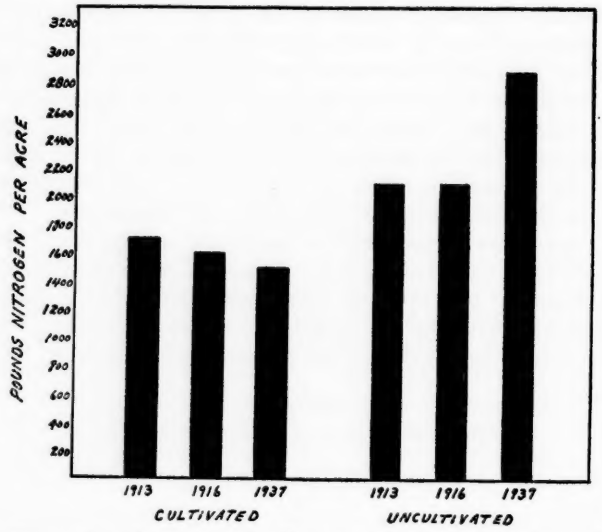

FIG. 1. NITROGEN IN CULTIVATED AND UNCULTIVATED SOILS AT DIFFERENT PERIODS

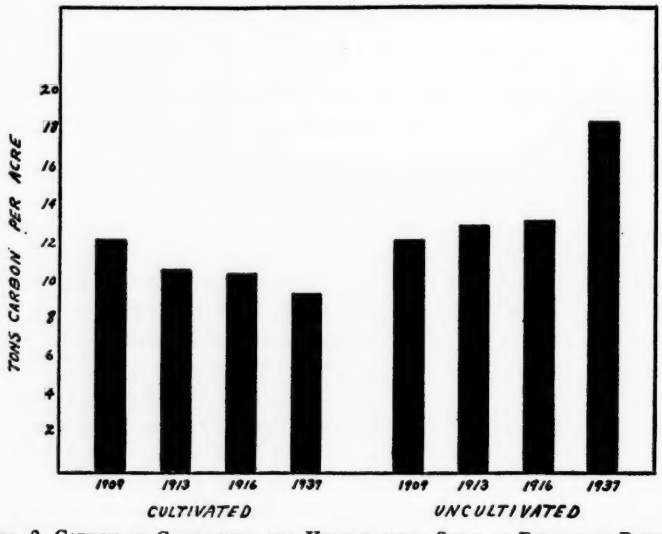

FIG. 2. CARBON IN CULTIVATED AND UNCULTIVATED SOILS AT DIFFERENT PERIODS

Table 2 shows the total nitrogen, carbon, phosphoric acid, and potash content of the surface soils for 1937 in comparison with these same constituents for the subsurface soils. It will be noted that the nitrogen and carbon con-

tent is lower for the subsurface soils than for the surface soils. Also the subsurface soils of the uncultivated section contain higher amounts of nitrogen and carbon than do the subsurface soils of the cultivated section. The total nitrogen and carbon content of both surface and subsurface soils of the uncultivated section is decidedly higher on the nitrogen-treated plots than on the no-nitrogen plots, but this does not hold true for the cultivated section.

Phosphoric acid and potash content

The surface soils of both sections of the plots contain more P_2O_5 than do the subsurface soils, undoubtedly because the major portion of soluble phosphates is fixed in the surface layer of soil (3). The plots that have received no phosphorus application are considerably lower in this constituent than are the other

TABLE 2

Analysis of soils collected in 1937 from cultivated and uncultivated plots

TREATMENT	CULTIVATED SECTION					UNCULTIVATED SECTION				
	pH	N	P_2O_5	K_2O	C	pH	N	P_2O_5	K_2O	C
		per cent	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Surface soils										
P and K	5.92	0.077	0.119	0.99	0.98	6.38	0.116	0.123	1.11	1.53
N and K	5.49	0.081	0.070	0.80	0.97	6.01	0.155	0.069	1.11	1.91
N and P	5.71	0.070	0.120	0.87	0.86	6.01	0.146	0.169	1.18	1.85
N, P, and K	5.79	0.080	0.131	1.17	0.98	6.29	0.132	0.156	1.16	1.70
Subsurface soils										
P and K	6.39	0.046	0.041	1.16	0.52	6.89	0.059	0.052	1.24	0.73
N and K	6.44	0.040	0.056	1.12	0.47	6.62	0.071	0.046	1.26	0.83
N and P	6.35	0.045	0.077	1.13	0.50	6.67	0.080	0.090	1.27	0.92
N, P, and K	6.50	0.049	0.083	1.13	0.53	6.83	0.069	0.084	1.16	0.85

plots. Soil from the phosphate-treated plots of the uncultivated section also shows a higher percentage of phosphoric acid than does soil from the corresponding plots of the cultivated section. This is no doubt due to the fact that on the uncultivated plots the crop residues are returned to the soil, whereas considerable P_2O_5 is removed in the crops grown on the cultivated section, and also because the loss through leaching is greater on the cultivated section.

The subsurface soils of both sections contain slightly more total potash than do the surface soils. This seems to be a characteristic of the soils of this region. The plots that receive no potash show, in most cases, just as high a percentage of this constituent as do the plots which receive potash, and in some cases even a higher percentage. This fact indicates that the normal potash content of the soil is high enough to mask the effect of applied potash, and, further, that there is little tendency for potash to accumulate in the soil.

It will be noted in tables 4 and 5, however, that the exchangeable potash is much lower on the plots receiving no potash treatment. The uncultivated section contains slightly more total potash than does the cultivated section. This may be due to the removal of some potash by the crops on the cultivated section and to greater losses through leaching.

Distribution of some soil constituents at different depths in the uncultivated plots

The data reported in table 3 were obtained from samples taken at depths of 0–2, 2–4, and 4–6 inches on the uncultivated plots. The pH values, which

TABLE 3

Distribution of some soil constituents at different depths in the uncultivated plots

TREATMENT	DEPTH	pH	N	P_2O_5	K_2O	C
	inches		*per cent*	*per cent*	*per cent*	*per cent*
P and K	0–2	5.78	0.169	0.201	1.209	2.266
P and K	2–4	6.15	0.124	0.164	1.217	1.592
P and K	4–6	6.30	0.102	0.139	1.198	1.214
Average			0.132	0.168	1.208	1.691
N and K	0–2	5.62	0.172	0.100	1.054	2.338
N and K	2–4	5.88	0.121	0.073	1.101	1.439
N and K	4–6	6.25	0.102	0.059	1.112	1.299
Average			0.132	0.077	1.089	1.692
N and P	0–2	5.22	0.193	0.195	1.450	2.573
N and P	2–4	6.07	0.123	0.156	1.306	1.480
N and P	4–6	6.50	0.095	0.122	1.380	1.194
Average			0.137	0.158	1.379	1.749
N, P, and K	0–2	5.77	0.184	0.171	1.116	2.409
N, P, and K	2–4	6.23	0.116	0.156	1.016	1.481
N, P, and K	4–6	6.40	0.100	0.140	1.116	1.189
Average			0.133	0.156	1.083	1.693

range from 5.22 to 6.50, increase progressively in each 2-inch layer. The higher pH values at the lower depth are undoubtedly due to the higher base saturation, which is reported in table 4.

The total nitrogen, carbon, and phosphoric acid, all progressively decrease with depth. The phosphoric acid tends to be fixed in the top 2-inch layer. The excess of the other constituents in the top 2 inches is due to an accumulation of the organic material. The plots receiving no phosphates show only about half as much P_2O_5 as do the other plots. Plots that receive no potash contain more total potash in the soil than do those that receive potash, a fact to which reference has already been made.

Comparison of the nature of the exchange complex in cultivated and uncultivated soils

The next phase of this investigation centers around certain changes which might be expected in the nature of the exchange complex existing in the soils of the uncultivated plots. The most important changes which might be looked for in this connection would be increases in the cation exchange capacity, and reductions in ultimate pH brought about by the variation in the ratio of the inorganic exchange complex to the organic exchange complex. The effect of the increase in the organic exchange complex can be evaluated by comparing the exchange capacities of the cultivated soils with those of the uncultivated soils.

TABLE 4

Distribution of exchangeable cations at different depths in the uncultivated plots

TREATMENT	DEPTH	pH	ULTIMATE pH	CATION EXCHANGE CAPACITY	UNSATURATION	EXCHANGEABLE CATIONS				ELECTRODIALYZABLE P_2O_5
						H	Ca	Mg	K	
	inches			*m. e. per 100*	*per cent*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*
P and K.....	0–2	5.78	3.92	11.46	36.7	4.21	5.91	0.48	0.86	4.37
P and K.....	2–4	6.15	4.06	9.90	30.2	2.99	5.94	0.41	0.56	3.23
P and K.....	4–6	6.30	4.09	8.36	32.9	2.75	4.53	0.44	0.64	2.10
N and K.....	0–2	5.62	3.85	9.72	71.1	6.91	2.84	0.42	0.55	0.49
N and K.....	2–4	5.88	4.03	7.57	37.1	2.81	3.54	0.31	0.91	0.35
N and K.....	4–6	6.25	4.13	7.09	37.7	2.67	3.62	0.28	0.52	0.26
N and P.....	0–2	5.22	3.91	12.06	55.2	6.66	4.59	0.51	0.30	4.46
N and P.....	2–4	6.07	4.02	9.36	31.7	2.97	5.79	0.32	0.28	3.14
N and P.....	4–6	6.50	4.15	8.11	22.8	1.85	5.53	0.32	0.41	2.40
N, P, and K.....	0–2	5.77	3.68	11.87	39.7	4.71	6.13	0.42	0.61	4.02
N, P, and K.....	2–4	6.23	4.20	8.98	32.2	2.89	5.27	0.35	0.47	2.90
N, P, and K.....	4–6	6.40	4.23	8.40	26.3	2.21	5.23	0.32	0.64	2.13

In table 4, data are reported for exchangeable bases, cation exchange capacity, unsaturation, and ultimate pH values of soils from the uncultivated section sampled at depths of 0–2, 2–4, and 4–6 inches. In all cases, the top 2-inch samples show a considerably greater cation exchange capacity than do samples from the lower levels. Thus, the top 2-inch sample has an exchange capacity of 11–12 m.e. per 100 gm. of soil, whereas the 4–6-inch sample has an exchange capacity of only a little over 8 m.e. per 100. The accumulation of new acidoid fractions, namely, humus and phosphoric acid, in the upper layers, accounts for this variation. The influence of phosphoric acid in this connection is reflected in a lower cation exchange capacity on the plots which receive no phosphate. The electrodialyzable phosphorus, shown in the last

column, also bears a relation to these facts, since it is present in largest amount (about 4 m.e. per 100) in the 0–2-inch depth, followed by that in the 2–4-inch depth, and finally by that in the 4–6-inch depth.

The ultimate pH values of these soils at different depths are further supporting evidence for the above reasoning. Thus, it will be noted that the ultimate pH values are lowest for those samples where there is the greatest accumulation of the acidoid fraction, namely, at the 0–2-inch depth. At this depth the ultimate pH values are all below 4.0.

The amount of exchangeable hydrogen and the percentage of unsaturation of these soils are always greatest in the top 2 inches and in most cases decrease progressively at the 2–4-inch and 4–6-inch depths. The decrease in exchangeable hydrogen with increasing depth accounts for the rise in field pH.

The exchangeable calcium does not vary to any significant extent in the different depths studied, on the plots that receive phosphates. On the plots which receive no phosphate, however, only about half as much exchangeable calcium was found as on the plots receiving phosphates. The exchangeable magnesium in these soils varies only slightly with the phosphate treatment. A slightly higher amount of exchangeable magnesium was found, however, in the top 2-inch samples.

The data for exchangeable potash vary significantly only when the plots receiving potash are compared with those receiving none. Approximately half as much exchangeable potash was found in the latter as in the former. This is interesting when compared with the total potash figures for the same plots which indicate a higher total potash content in the plots receiving no potash.

Table 5 presents data on the cation exchange capacity, unsaturation, and exchangeable bases from soils of the cultivated and uncultivated plots for both surface and subsurface samples. The cation exchange capacities of the soils from the cultivated plots are all lower than those from the respective uncultivated plots. This fact, of course, can be attributed mainly to the lower humus content of the soils from the cultivated section where the organic matter has undergone oxidation. The subsurface soils also have lower cation exchange capacities than the surface soils, since the organic matter and phosphoric acid content of these subsurface soils are lower. With one exception, the plots which receive no phosphate are the lowest in cation exchange capacity both in the surface and subsurface soils. This is to be expected, since phosphate is one of the acidoids which bring about an increase in the cation exchange capacity (7, 8).

The surface soils from both sections are decidedly more unsaturated than the subsurface soils, a fact which is further substantiated by the pH values and by the data on exchangeable hydrogen. Furthermore, the surface soils from the cultivated plots are considerably more unsaturated than those from the uncultivated section. The effects of leaching and of removal of bases by the crops on the cultivated section are responsible for this condition. Thus,

it is interesting to note that both exchangeable calcium and magnesium are lower on the cultivated plots than on the uncultivated.[2] The especially low value for exchangeable calcium (1.80 m.e. per 100) on the plot receiving no phosphate (cultivated section) is associated with the low cation exchange capacity of this plot. On the uncultivated section, the exchangeable calcium of this plot has been increased to 4.95 m.e. per 100, because of the increase in the amount of organic exchange complex as indicated by the higher carbon

TABLE 5

Exchangeable cations from cultivated and uncultivated plots

TREATMENT	CULTIVATED SECTION							UNCULTIVATED SECTION						
	pH	Cation exchange capacity	Unsaturation	Exchangeable cations				pH	Cation exchange capacity	Unsaturation	Exchangeable cations			
				H	Ca	Mg	K				H	Ca	Mg	K
		m. e. per 100	*per cent*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*		*m. e. per 100*	*per cent*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*	*m. e. per 100*
Surface soils														
P and K	5.92	6.23	45.3	2.87	2.57	0.53	0.26	6.38	8.56	17.3	1.49	5.43	0.88	0.77
N and K	5.49	5.53	49.2	2.49	1.80	0.52	0.20	6.01	9.03	27.0	2.46	4.95	0.86	0.77
N and P	5.71	5.96	39.2	2.34	3.04	0.52	0.07	6.01	10.27	29.0	2.98	5.99	1.01	0.29
N, P, and K	5.79	6.71	55.9	3.74	2.27	0.48	0.22	6.29	9.91	23.0	2.29	5.80	0.95	0.87
Subsurface soils														
P and K	6.39	4.10	17.0	0.67	2.57	0.56	0.30	6.89	5.82	3.7	0.22	4.40	0.60	0.66
N and K	6.44	3.16	6.6	0.21	2.18	0.53	0.24	6.62	5.35	1.5	0.09	3.89	0.67	0.74
N and P	6.35	4.48	29.3	1.30	2.52	0.60	0.05	6.67	6.70	13.9	0.94	4.95	0.60	0.23
N, P, and K	6.50	4.64	10.0	0.46	3.29	0.62	0.26	6.83	6.30	7.0	0.45	3.94	0.70	0.71

content (see table 2). The new complex, therefore, retains the exchangeable calcium to a greater degree than does the exchange complex on the cultivated section. It is also true that there is less exchangeable calcium removed from the uncultivated section. The subsurface soils have approximately the same amount of exchangeable calcium as do the surface soils in the cultivated section, even though the cation exchange capacity of the former is lower. This fact together with the unsaturation and the pH values indicates a movement of lime to the lower depths.

The exchangeable potash, like the calcium and magnesium, is considerably

[2] The values for exchangeable magnesium reported in table 5 are twice, and in some cases three times, as great as the values reported for corresponding plots in table 4. This may be explained by the fact that the values reported in table 5 were obtained by extraction with normal ammonium acetate, whereas those in table 4 were obtained by electrodialysis. It has been shown (10) that electrodialysis does not remove all the exchangeable magnesium.

less on the cultivated plots than on the uncultivated, and for similar reasons. Again it may be observed that the plots receiving no potash contain only about one-third as much exchangeable potash as do the other plots in the cultivated section. Relatively more exchangeable potash is present in the uncultivated section than in the cultivated section because the retention of potash in the exchange complex has been enhanced by the greater exchange capacity of these plots. Furthermore, but little potash has been removed from this section by crops (only one crop removed).

From the facts presented, one might well infer that land which has been taken out of cultivation and abandoned to natural grasses and weeds gradually gains in fertility mainly through accumulative processes. Although these processes are very slow, the accumulation of organic residues brings about a greater cation exchange capacity for the soils, which if fertilized will then retain more bases in the exchange complex.

SUMMARY

Changes in the chemical composition and physicochemical properties of soil from cultivated land and land abandoned to grass and weeds over a period of 30 years are reported.

Samples of soil were collected in 1913, 1916, and 1937. Subsurface samples ($6\frac{2}{3}$–12 inches) were taken in 1937, and, in addition, four plots from the uncultivated section were sampled at successive 2-inch depths down to 6 inches.

Total nitrogen and carbon determinations were made on all samples; and, in addition, total phosphoric acid and potash determinations were made on the 1937 samples. Base exchange studies were also carried out on the 1937 surface and subsurface samples and on the special samples collected at depths of 0–2, 2–4, and 4–6 inches.

Without exception, both nitrogen and carbon are now higher in the uncultivated section than in the cultivated. These data are in accord with the view that liberal applications of minerals favor the appearance of volunteer leguminous plants on abandoned land and also the fixation of atmospheric nitrogen by nonsymbiotic bacteria. On the other hand, the cultivated sections show a gradual depletion of their supply of nitrogen and carbon, since there is comparatively little encouragement for volunteer leguminous plants to come in and restore nitrogen losses, and the removal of the crops prevents the maintenance of the organic matter supply.

Although the subsurface soils of both sections show lower percentages of both nitrogen and carbon than do the surface soils, the subsurface soils of the uncultivated section show higher percentages of these elements than do the subsurface soils of the cultivated section.

Where phosphorus and potassium have been applied, soil from the uncultivated section shows a slightly higher percentage of these elements than does soil from the corresponding plots of the cultivated section. In all plots, phos-

phorus is higher in the surface soil than in the subsurface soil, but the reverse is true for potash.

In the uncultivated section, the pH values increase progressively with each 2-inch increase in depth, down to 6 inches; and the total nitrogen, carbon, and phosphoric acid decrease progressively. The higher pH values at the lower depths are attributed to the higher base saturation at these levels. Much of the phosphoric acid was found to be fixed in the top 2-inch layer; the larger amounts of the other constituents found in this layer are due largely to an accumulation of organic matter.

A study was made of the nature of the exchange complex in the cultivated and uncultivated soils. The cation exchange capacity of the uncultivated soils, which was greatest in the top 2 inches, was found to be higher, in every instance, than that of the cultivated soils. The accumulation of the acidoid fraction, namely, humus and phosphoric acid, in the upper layers of the uncultivated section account for this variation. Furthermore, the ultimate pH values are lowest on those soils in which there is the greatest accumulation of the acidoid fraction.

The effect of leaching and of removal of exchangeable bases by cultivation is reflected in the lower amount of exchangeable calcium, magnesium, and potash in the cultivated soils than in the uncultivated soils.

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ACTION OF HYDROGEN PEROXIDE ON WEATHERED MICA

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It was noted, while a mechanical analysis was being made of a soil from the Mojave desert, California, that the coarse separates of the lower horizons seemed to contain much more mica than did the original soil. The character of the mica also was strikingly different after the mechanical analysis. The dark brown or black particles of the mica were transformed into a golden flaky variety.

Investigation revealed that the change in the mica was due to the hydrogen peroxide treatment given the soil to destroy organic matter before the mechanical analysis was made. Tablets of the dark colored mica were picked out of the weathered granite from which the soil was derived and were treated with a few cubic centimeters of 30 per cent hydrogen peroxide. Immediately the peroxide decomposed around the mica particles. Then gradually the mica began to exfoliate, and in a few minutes a golden flaky mass of thin mica plates was present in suspension, most of the flakes rising to the surface. Plate 1 shows the mica before and after exfoliation. On oven drying, the flakes formed large coherent masses. Pieces of the untreated mica, when heated, expanded considerably. This fact, along with the optical properties and an X-ray analysis of the material, identified it as an altered hydrated mica having some properties of the vermiculites, which are known to have spacing between the plates. It seemed reasonable, therefore, to suppose that the peroxide penetrated the mica plates, that something there caused its decomposition, and that the evolution of the oxygen split apart the plates. Samples of unweathered biotite and muscovite, which have a compact structure, were tested, but they did not react with the peroxide.

About 20 different samples of vermiculites were then obtained from the Mineralogy Division of the National Museum to be tested. Exfoliation with very active decomposition of the peroxide occurred with only two of the samples, both of them varieties of philadelphite; several others caused a slow decomposition of the peroxide with slight exfoliation; but most of them did not react at all.

The philadelphite samples appeared to be somewhat weathered, as did the soil mica, whereas the other vermiculites appeared unweathered. It was suspected, therefore, that some alteration product between the plates acted as a catalyst in the decomposition of the peroxide. As manganese dioxide is a

very active catalyst and as manganese is a common constituent of micas, a test for that element was made. The two samples of philadelphite, the mica from the Mojave desert soil, and two samples of the vermiculites reacting weakly with the peroxide were digested in 4 per cent nitric acid for about an hour on the steam bath. To all appearances the samples were not altered materially by this treatment. Aliquots of the solutions were then tested for manganese. Very strong test for manganese were obtained in the soil mica and in both samples of philadelphite, whereas only a slight test was obtained in the other two vermiculites. The parent material of the Mojave desert soil had a manganese content of 0.07 per cent, and selected pieces of the altered mica contained 0.31 per cent. It seemed clear, then, that it was manganese dioxide between the mica plates which caused the reaction. As further confirmation of this, a sample of weathered mica was obtained from a rock exposure in Rock Creek Park, Washington, D. C. Small pieces of this mica readily broke off when treated with hydrogen peroxide. But the exfoliation was in small particles rather than in complete plates. From these pieces a thin film of manganese dioxide could be scraped from the surface of the mica plates.

It should be mentioned that with some weathered micas and vermiculites, organic matter or other catalysts such as nickel oxide or chromium oxide, may be present between the plates and cause the same reaction. Hydrated ferric oxide, also, is moderately active in decomposing hydrogen peroxide and may be the effective agent in some instances in causing exfoliation.

Alkaline solutions of hydrogen peroxide decompose much more readily than acid solutions. A few drops of ammonium hydroxide added to the vermiculite samples which reacted weakly with the peroxide caused a more vigorous reaction accompanied by exfoliation of the material. A similar acceleration of the reaction was obtained by heating the slightly acid solution.

The following comments may be made in connection with this reaction: It gives evidence of the presence of manganese dioxide between the plates of some hydrated weathered micas. This suggests the similar occurrence of metallic oxides such as nickel or chromium in altered micas containing these metals. Thus some indication may be obtained concerning the structure of altered micas and the nature of the weathering.

The exfoliation of weathered mica with hydrogen peroxide may provide a simple means of separating the mica from other minerals with which it may occur. The thin flakes can be easily floated off in water while the other minerals sink quickly to the bottom of the vessel. This scheme was used successfully with the coarse fractions of the Mojave desert granite soil.

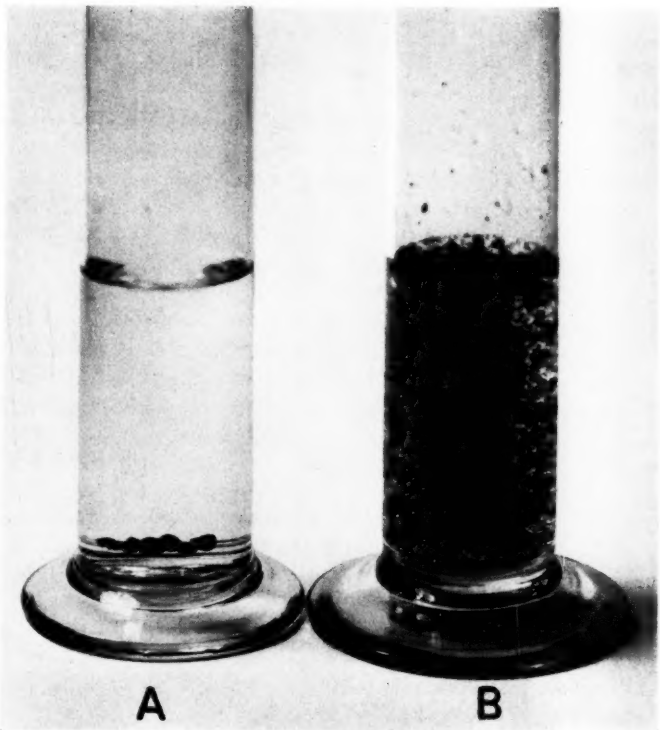
A third consideration is the effect of the peroxide on the mechanical analysis of soils high in mica. Two distinct effects are noted when 6 per cent hydrogen peroxide is used as a pretreatment for removing organic matter in the Bureau of Chemistry and Soils mechanical analysis procedure. In case of a soil containing the hydrated type of weathered mica, treatment with peroxide

resulted in an increase in the coarse separates and a consequent decrease in the finer fractions. This was due to the cohesion of the exfoliated plates of mica when the soil was heated after the peroxide treatment. In case of a soil containing weathered mica not of the hydrated type, the opposite effect was obtained. The hydrogen peroxide tended to break up the larger pieces of mica into smaller flakes, thereby increasing the finer fractions and consequently decreasing the coarser fractions. Significant differences due to the peroxide treatment in the mechanical analysis would be expected only in soils high in mica, especially in the lower horizons where the mica particles are coarser and more abundant.

PLATE 1

MICA PARTICLES IN WATER COMPARED WITH SIMILAR PARTICLES IN 6 PER CENT HYDROGEN PEROXIDE SOLUTION

A, untreated pieces of mica; *B*, mica after exfoliation with hydrogen peroxide.


A
B

RAPID DETERMINATION OF ORGANIC CARBON IN SOIL[1]

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The usual procedure for determining organic carbon in soil is to determine total carbon by a combustion method and to deduct from that finding the inorganic carbon, determined separately. This procedure is slow and tedious and requires special apparatus. As far as the author can ascertain, no satisfactory method for determining organic carbon in soil directly has been proposed. A titration method which depends on measurement of the amount of chromic acid reduced by the soil organic matter has been worked out (4), but it seems not to account for all the carbon.

The direct method for determining organic carbon in vegetative plant tissue (3) is so rapid and accurate for carbohydrate and protein material that it was decided to try it on soils. At first it was thought that soil minerals would interfere with the color. Several types of soil were stripped of organic matter, and it was found that the dissolved minerals in strong sulfuric acid did not impart enough color to cause significant off-color tints in the reading of the brown carbon color in a colorimeter. It may be that some soils not tried by the author would give color interference, but it seems unlikely that this would occur in the common soil types. As very few fatty compounds or aromatic compounds not attacked by sulfuric acid are present in soils, the method should be very accurate in determining total organic carbon under usual field conditions.

PROCEDURE

Heat strongly on a hot plate (not over a free flame) 0.25 to 2 gm. (depending on the carbon content) of soil in 25 cc. of 50 per cent (by volume) sulfuric acid in a 200-cc. Erlenmeyer flask until foaming ceases, white fumes start coming off profusely, and acid just starts to condense on the sides of the flask but not longer than this. Cool, make up to 25 or 50 cc. (depending on the C content) with 50 per cent H_2SO_4. Shake the solution well, pour into a tall graduate or test-tube, and allow to settle. As soon as the supernatant liquid is clear, compare with the standard in a colorimeter. Some solutions may have to stand overnight to clear sufficiently for satisfactory reading. If it is desired to make immediate reading, a plug of glass wool may be placed in an appropriate funnel and the solution decanted from the soil residue as much

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

as possible on to the filter. Considerable filtrate should be allowed to run back into the original flask. Pour back until the solution is clear.

PREPARATION OF STANDARD

Heat on a hot plate 5 cc. of 0.25 per cent pure anhydrous glucose solution with 25 cc. of 50 per cent (by volume) H_2SO_4 to the same point as that in the soils procedure. Care must be taken not to heat too long, or the standard will be weak. The volume will now be about 15 cc. Cool, and make up to exactly 20 cc. with 50 per cent H_2SO_4. One cubic centimeter contains 0.25 mgm. of C. If 0.125 mgm. is desired for low C soils, dilute to 40 cc. with 50 per cent H_2SO_4. The standard should be made up fairly close to the time the unknowns are tested.

TABLE 1

Organic carbon, in per cent

	ALIQUOT	SAMPLE 1	SAMPLE 2
Black silt loam	1	3.09	3.06
	2	3.06	3.03
Black silt loam and millet turned under	1	3.55	3.47
	2	3.54	3.40
Red clay	1	1.25	1.20
	2	1.23	1.18
Soil below sod	1	2.23	2.19
Black silt loam and 20 tons of manure per acre	1	4.46	4.56
Black silt loam and 40 tons of manure per acre	1	6.40	6.41

RESULTS

At first several soils were tested to see how the test behaved with different types and in different samplings of the same soil. The results are presented in table 1.

In order to check the method further, additions of known quantities of carbohydrate were used. The results, presented in table 2, show that virtually all the added carbon was recovered.

In order to make certain that all carbon was being determined, samples of soil in which organic carbon had been accurately determined were obtained from the University of Illinois and from the U. S. Department of Agriculture. Table 3 presents these results.

It will be seen from this table that the colorimetric method gave slightly higher results in nearly all cases. An explanation might be that the standard was slightly weak or that it or the soil samples had absorbed moisture. To test this, a U. S. Bureau of Standards sample was dried at 55°C. for several days. The results were the same. Overheating in the acid digestion, of

TABLE 2

Recovery of added organic carbon in soil

SAMPLE*	MILLIGRAMS OF CARBON		SAMPLE*	MILLIGRAMS OF CARBON	
	Found	Recovered		Found	Recovered
1 c	19.840	4.96	5 c	19.840	4.96
1	14.880		5	14.880	
2 c	19.230	5.03	6 c	21.185	4.95
2	14.205		6	16.235	
3 c	18.940	4.57	7 c	19.685	4.98
3	14.370		7	14.705	
4 c	25.000	4.84	8 c	19.685	4.98
4	20.160		8	14.705	
1 c R.C.	16.500	4.60	9 c	27.175	4.86
1	11.900		9	22.320	
2 c R.C.	17.100	5.20	10 c	37.000	4.95
2	11.900		10	32.050	

* 500-mgm. samples of soil were used, except for red clay (R.C.), 1000-mgm. samples of which were used; 5 mgm. of carbon in the form of pure glucose was added to each "c" sample.

TABLE 3

Comparison of rapid colorimetric method for total carbon with furnace combustion method

SAMPLE*	TOTAL ORGANIC CARBON		SAMPLE‡	TOTAL ORGANIC CARBON	
	Furnace combustion†	Colorimetric		Furnace combustion	Colorimetric
	per cent	*per cent*		*per cent*	*per cent*
13873	3.13	3.20	1. Barmea	0.53	0.54
13876	1.98	2.11	2. Roseworthy	2.91	2.95
13915	2.38	2.42	3. Medemblik	0.80	0.80
14035	1.46	1.66	4. Ohio	17.83	17.99
14076	1.22	1.38	5. Mnyusi	2.72	2.77
13777	4.11	4.31	6. Ohehe	2.74	2.80
13780	1.66	1.72	7. Hungarian	2.36	2.42
14034	2.53	2.55	8. Hungarian		
14052	2.27	2.31	(B horizon)	1.86	1.92
14075	2.35	2.45	9. Iowa	2.07	2.23

* Furnished by R. H. Bray, agronomy department, University of Illinois; see (1) for description.

† See Winters and Smith (5).

‡ Furnished by H. G. Byers, Bureau of Soils, U. S. Department of Agriculture; see (2) for description.

course, might weaken the standard; but duplicate standards checked, which probably would not be so if this were a source of error. As differences between findings by the two methods are not large, the rapid colorimetric method appears to be fairly accurate. High amounts of sodium chloride in some of the U. S. Department of Agriculture samples did not interfere. In fact, none of the soil extremes tested appeared to cause interference. Some were a little harder to clear than others, but the brown color matched well with the standard in all samples.

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INTERACTION BETWEEN CARBONATES AND SOILS

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One of the most characteristic reactions of acids is associated with their power of decomposing carbonates with the evolution of CO_2. On the other hand, alkali carbonates can decompose alkaline earth salts with the production of insoluble carbonates and the alkali salt of the acid. Carbonates play an important part in soils. The amelioration of acidity by the application of $CaCO_3$ and the deterioration resulting from the introduction and accumulation of Na_2CO_3 are well known examples of the rôle of carbonates in soils.

Titration curves of alkali carbonates with acids would furnish an excellent method of following these reactions, for the production of CO_2 could be estimated at every step by determining the pH value of the reaction mixture. This is obvious from the fact that the titration of an alkali carbonate with any acid is equivalent to its titration with CO_2 up to the formation of bicarbonate. Beyond that stage the bicarbonate begins to decompose, and the characteristic shape of the curve is dependent on the nature of the acid used, for with a strong acid the inflection will be sharper than that with a weak acid, on account of the hydrolysis of the alkali salt. These titration curves would not only furnish information on the decomposition of carbonates but also indicate the strength of the acid.

On the other hand, titration curves of alkali carbonates with alkaline earth salts can give valuable information on the precipitation of insoluble carbonates. Apparently such curves would correspond to the pH values of carbonate solutions of decreasing concentrations until all the carbonate was precipitated, when the pH value would show a sudden drop.

Since carbonates are known to react not only with acid soils but with soils containing exchangeable Ca (2), similar methods of study could be employed as with ordinary acids and soluble Ca-salts. Two types of alkali and alkaline earth carbonates could be studied, of which Na_2CO_3 and $CaCO_3$ are typical and generally found in soils.

In the experiments reported in this paper, the following reactions were studied:

Reaction of Na_2CO_3 with common soluble acids, with soil acidoid, and with an insoluble acid.

Reaction of Na_2CO_3 with calcium salts and with calcium soils.

Reaction of $CaCO_3$ with common soluble acids and with soil acidoids.

Reaction of $CaCO_3$ with sodium salts and with sodium soils.

Exchangeable bases were removed from soils in the usual way by exhaustive treatment with dilute HCl. From the resulting H-soils, Ca- and Na-soils, were prepared by neutralizing with the corresponding hydroxides.

REACTION OF SODIUM CARBONATE WITH ACIDS AND WITH SOIL ACIDOID

Sodium carbonate is the sodium salt of dibasic carbonic acid. Titration with an acid first leads to the formation of the acid salt. The most characteristic feature of this titration is the point of inflection corresponding to the acid salt formation. The stronger the acid used for titration, the more pronounced is this point of inflection. This is shown by the titration curves with various acids and with soil acidoid (fig. 1). It is to be understood, of course, that the soil was used as a whole. Throughout this work no attempt was made to

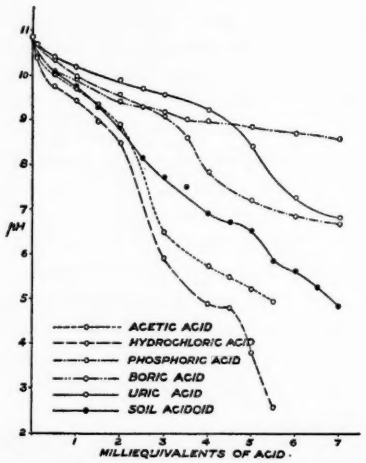

FIG. 1. TITRATION OF SODIUM CARBONATE WITH VARIOUS ACIDS AND WITH SOIL ACIDOID

(Na_2CO_3 = 5 me.)

separate the clay fraction, for such a procedure is unnecessary and is likely to confuse the issue. Colloidal acids are so uniformly distributed in the soil that no advantage can be gained by separating them from the inert material. The titration method of determining the equivalent weight of a soil acidoid ($T/2$) has been explained (3). All the incremental additions of soil acidoids or of Ca soil are given, therefore, in milliequivalents; thus, all the results are brought to a uniform basis. Figure 1 shows that the point of inflection corresponding to the bicarbonate formation is as pronounced with the soil acidoid as that with acetic acid or uric acid, and more prounounced than that with boric acid. The case of uric acid requires special mention. This acid is virtually insoluble in water, the solubility being 1:10,000. Since a normal solution could not be made, uric acid was added in the solid state, the exact

amount corresponding to 1 m.e. being weighed for each incremental addition. It would thus appear that the insoluble part of uric acid behaves as an acidoid and gives a titration curve with sodium carbonate as if it were soluble. These results emphasize the fact that the distinction between ordinary acids and soil acidoids is purely arbitrary and merely refers to the differences in their solubility.

REACTION OF SODIUM CARBONATE WITH CALCIUM SALTS AND WITH CALCIUM SOILS

Calcium salts react with Na_2CO_3, with the production of $CaCO_3$, which is insoluble. The titration of a Na_2CO_3 solution with a calcium salt, therefore, represents the pH value of Na_2CO_3 solutions of decreasing concentrations, until the whole is precipitated as $CaCO_3$, when there is a sudden drop in the pH

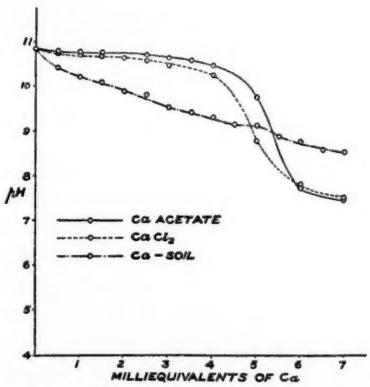

FIG. 2. TITRATION OF SODIUM CARBONATE WITH CALCIUM SALTS AND WITH CALCIUM SOIL (Na_2CO_3 = 5 m.e.

value. The pH value again shows only a slight change with further additions of Ca salt. The drop corresponding to the precipitation of the entire Na_2CO_3 is considerable with neutral salts like $CaCl_2$, it is less with salts of weak acids like Ca acetate, and it may be entirely absent with salts of extremely weak acids. This will be clear from the following considerations: Salts of strong acids like $CaCl_2$ or NaCl are unbuffered neutral solutions, therefore they take up the pH value of any buffer solution to which they are added. Salts of weak acids, on the other hand, are more buffered than are the neutral salts, and their titration curves with strong bases generally do not show a sharp inflection at the neutral point. The latter also lies at a higher pH value. Sodium carbonate, a salt of a strong base with a weak acid, is thus well buffered. When $CaCl_2$ is added to it, the products of reaction are $CaCO_3$, which is insoluble, and NaCl, which has very little effect on the reaction, and thus a sharp

inflection is obtained when the whole of the Na_2CO_3 has been precipitated as $CaCO_3$. The curves showing the course of reaction between Na_2CO_3 and calcium salts and calcium soil are given in figure 2. It is seen that the soil curve is steeper than the salt curves but shows no inflection. The reason for this apparently anomalous behavior is that the pH value of the soil was about 6.5. As this does not correspond to the neutral point, the soil could still react with sodium carbonate (2). The pH value is thus lowered still further until the whole of the sodium carbonate has reacted. The completion of this reaction, however, leaves the soil at a higher pH value than would be the case with a neutral salt. Further additions of the soil amount to adding one buffer solution to another, and the drop in pH, therefore, is comparatively slight.

REACTION OF CALCIUM CARBONATE WITH ACIDS AND WITH SOIL ACIDOIDS

In view of the abundance of $CaCO_3$ in soils and of its use for neutralizing soil acidity, the reaction of $CaCO_3$ with acids and with soil acidoids is of particular interest. The well-known reaction

$$CaCO_3 + 2\ HR \rightarrow CaR_2 + H_2O + CO_2,$$

in which $CaCO_3$ is decomposed by acids with the production of CO_2, unlike the reaction with alkali carbonates, has no point of inflection corresponding to the bicarbonate formation. The very slight solubility of $CaCO_3$ in water has an effect on the course of the titration curve similar to the effect of Ca salts on the reaction of a saturated solution of $CaCO_3$, until all the $CaCO_3$ is used up. The point at which this occurs will correspond to the inflection of the curve, if the titration is done with a strong acid; the inflection will be less marked with a weak acid and may be entirely absent with an extremely weak acid. The titration curves of $CaCO_3$ with common acids and soil acidoids are given in figure 3. It will be seen that the inflection with the soil acidoid is at least as marked as that with acetic acid.

It was of interest to determine the final pH values of acidoids from a number of soils when shaken with excess of $CaCO_3$. For this purpose, 19 soils after the usual acid treatment and leaching were shaken for 1 hour and for 48 hours with excess of $CaCO_3$; the final pH values were determined by the glass electrode; and the amount of $CaCO_3$ decomposed by the soil was estimated by the carbonate-oxalate method of Puri (2). The results are given in table 1. Base exchange capacities of the soils ($T/2$ values), determined by titration (3) and by ammonia that can react with the soils (4), are also given. It will be noted that 48 hours' shaking is not necessary for the attainment of equilibrium. There is no appreciable increase in the Ca taken up by the soil when shaken longer than 1 hour, though there is a slight fall in the pH values. The general agreement between $T/2$ values, the ammonia that reacted, and the equilibrium with $CaCO_3$ measured under entirely different sets of conditions would leave no doubt that we are dealing in all cases with reactions that are fundamentally

identical. This can be reconciled only with the view that acidoids behave like true acids in their action on carbonates, ammonia, and other alkalies. It is seen that the pH value of soil in equilibrium with $CaCO_3$ is approximately equal to 7, in spite of the fact that a saturated solution of $CaCO_3$ has a pH value of 8.5. The fact that this value is reduced to 7 in the presence of Ca-soil is of great interest. It is evidently due to the lowering of the dissociation of $CaCO_3$ by the presence of a common ion; and the action is similar to that of soluble Ca salts. This will be seen from figure 4, in which the pH values of a saturated solution of $CaCO_3$ in the presence of varying amounts of Ca and Na salts and soils are given. The influence of single-base soils is of the same order as that of acetates.

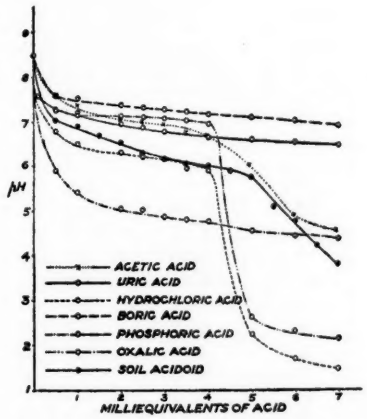

FIG. 3. TITRATION OF CALCIUM CARBONATE WITH VARIOUS ACIDS AND WITH SOIL ACIDOID ($CaCO_3$ = 5 m.e.)

The method of finding the lime requirement of acid soils based on the reaction with $CaCO_3$ has been advocated by several workers (1, 5). The fact that the final pH value corresponds to the neutral point would give added weight to the method.

It appeared of interest to determine the titration curve of a soil with $CaCO_3$ and also to find out whether a soil, after being partly neutralized with $Ca(OH)_2$ or NaOH, would take up from $CaCO_3$ the remaining portion of Ca to correspond to the equilibrium point. Increasing amounts of $Ca(OH)_2$, $Mg(OH)_2$, and NaOH were added to a soil, and the pH of each mixture was determined after 48 hours' shaking. These suspensions were then shaken with excess of $CaCO_3$; and the final pH value, as well as the Ca taken up by the soil, was determined in every case. The results are given in table 2.

A close examination of table 2 reveals some interesting facts. It is seen that when the soil acidoids are first neutralized with NaOH and then shaken

with $CaCO_3$, the total amount of exchangeable bases, that is, Na plus Ca, remains the same, and the soil is able to take up small amounts of Ca even at pH 9.68. This is possibly due to replacement of a certain amount of Na by Ca. The pH values of the single-base soils were determined before and after $CaCO_3$ treatment, and it is worthy of note that though the pH value of the H-soil brought to equilibrium with $CaCO_3$ is approximately 7, sodium and calcium soils at higher pH values can still take up some Ca from $CaCO_3$. The total bases at the equilibrium point are higher in the Na-soil than in the Mg-

TABLE 1

Exchangeable calcium in H-soils shaken with calcium carbonate

SOIL NUMBER, M SERIES	1 HOURS' SHAKING		48 HOURS' SHAKING		NH_3 TAKEN UP BY 100 GM. SOIL	$T/2$ PER 100 GM. SOIL
	pH in equilibrium with $CaCO_3$	Exchangeable calcium per 100 gm. soil	pH in equilibrium with $CaCO_3$	Exchangeable calcium per 100 gm. soil		
		m.e.		*m.e.*	*m.e.*	*m.e.*
1	7.12	25.2	6.78	24.4	24.4	23.2
2	7.28	13.2	6.86	16.0	11.6	12.0
3	7.24	15.2	6.79	13.6	13.4	14.6
4	7.29	13.2	6.78	14.4	11.4	12.8
5	7.18	22.8	6.76	21.6	18.6	20.0
6	7.30	10.8	6.90	9.2	9.2	10.0
7	7.25	17.2	6.81	16.8	13.6	28.0
8	7.20	26.8	6.74	25.2	26.2	28.2
9	7.31	8.4	6.99	7.2	8.8	12.4
10	7.20	21.6	6.90	20.0	20.4	16.6
11	7.33	18.0	6.93	14.0	13.0	14.0
12	7.24	19.2	6.88	19.6	19.8	16.24
13	7.37	8.4	6.97	9.2	9.6	9.44
14	7.26	14.8	6.93	15.2	10.6	10.64
16	7.43	7.6	7.00	8.8	6.2	5.84
17	7.32	8.4	6.92	10.4	13.6	10.0
21	7.25	15.2	7.04	14.8	22.6	14.24
22	7.34	9.2	7.01	13.6	12.0	12.32
23	7.28	12.0	6.95	10.0	14.4	12.48

soil or in the Ca-soil. The mutual salt effect of $CaCO_3$ and Ca-soil, each suppressing the ionization of the other, is well brought out by these results.

Another noteworthy fact is that exchangeable Ca in a soil containing exchangeable Mg can be determined by the K_2CO_3-acetate-oxalate method of Puri (2). This would seem extraordinary, in view of the fact that Ca and Mg salts are generally precipitated together by oxalates. The reason for this separation in soils lies in the fact that the activity coefficient of a Ca-soil is very much higher than that of a Mg-soil; the latter is therefore extremely insoluble and does not react with potassium oxalate within the time prescribed for this reaction, that is, 15 minutes.

The lime status of a soil (5) is defined as the ratio expressing as percentage

the exchangeable Ca actually present in the soil (Ca_1) over the exchangeable Ca the soil will have when brought into equilibrium with $CaCO_3$ (Ca_2), that is,

$$\text{L. S.} = 100\ Ca_1/Ca_2$$

It must be pointed out that this ratio can have significance only when exchangeable bases other than Ca are absent, a condition scarcely met with in

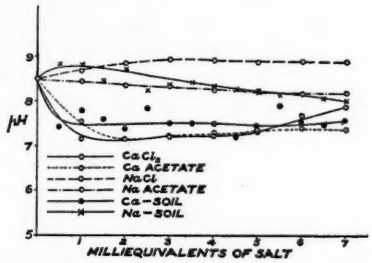

FIG. 4. INFLUENCE OF CALCIUM AND SODIUM SALTS AND OF CORRESPONDING SOILS ON THE pH VALUE OF CALCIUM CARBONATE SUSPENSION

($CaCO_3$ = 5 m.e.)

TABLE 2

Exchangeable calcium in H-soil first partly neutralized with NaOH, $Mg(OH)_2$, and $Ca(OH)_2$ and then shaken with $CaCO_3$

SODIUM HYDROXIDE				MAGNESIUM HYDROXIDE				CALCIUM HYDROXIDE				CALCIUM CARBONATE			
Amount 0.1 *N* NaOH, in cc.	pH	pH after shaking with $CaCO_3$	Exchangeable Ca, in cc. 0.1 *N* per 5 gm. of soil	Amount 0.1 *N* Mg $(OH)_2$, in cc.	pH	pH after shaking with $CaCO_3$	Exchangeable Ca, in cc. 0.1 *N* per 5 gm. of soil	Amount 0.1 *N* $Ca(OH)_2$, in cc.	pH	pH after shaking with $CaCO_3$	Exchangeable Ca, in cc. 0.1 *N* per 5 gm. of soil	Amount 0.1 *N* $CaCO_3$, in cc.	pH	pH after shaking with $CaCO_3$	Exchangeable Ca, in cc. 0.1 *N* per 5 gm. of soil
0	3.46	7.15	32.2	0	3.46	7.15	32.2	0	3.46	7.15	32.2	0	3.46	7.15	32.2
5	4.53	7.20	30.0	5	3.65	7.09	22.8	5	3.58	7.10	33.6	5	3.67	7.08	28.4
10	4.94	7.34	26.5	10	4.07	7.15	20.8	10	3.86	7.13	33.0	10	3.76	7.08	34.2
20	6.40	7.66	14.6	15	4.63	7.19	17.0	15	4.58	7.18	32.6	20	5.54	7.06	32.8
25	7.68	7.88	8.6	20	6.10	7.30	12.0	20	5.97	7.26	32.2	25	6.75	7.06	34.2
30	9.06	8.54	3.6	25	7.24	7.56	6.8	25	6.94	7.36	33.0	30	7.02	7.04	33.4
35	9.68	9.04	1.2	35	7.80	7.62	3.6	30	7.50	7.33	34.2	35	7.04	7.12	34.4
40	10.20	9.80	0.6	40	7.97	7.68	3.0	35	8.58	8.28	39.4	40	7.07	7.12	31.0
50	10.87	10.34	0.4	50	8.25	7.78	1.6	40	9.14	8.82	40.6	50	7.15	7.08	34.4
..	.....	.....	...	60	8.46	8.03	2.0	50	9.68	9.50	41.4	..	....	....	....
..	.....	.....	...	70	8.83	8.12	1.4	..	....	....	....	..	....	....	....

natural soils. A word of caution, therefore, must be sounded in connection with the interpretation of this constant when different soils are compared. Lime status as a ratio also implies a straight line relationship between the Ca present in the soil and the maximum Ca the soil can take up from $CaCO_3$.

This is correct only within a restricted range of pH, as will be seen from the titration curve of a base-free soil with $CaCO_3$ (fig. 5). For practical purposes this range may be quite sufficient, but the inherent defects of this ratio as a fundamental soil constant must be borne in mind if a proper perspective of the true state of affairs is to be maintained.

From the practical point of view, much may be said in favor of the $CaCO_3$ equilibrium point as the basis for determining lime requirement of soils. The very slight solubility of $CaCO_3$ is a great advantage, because a saturated solution is maintained and the experimental conditions can be easily reproduced, thus leading to a greater uniformity of results in the hands of different workers. It is necessary, however, to exercise judgment in the use of this method. It is evident that even soils requiring no lime might show lime requirement if the method were used indiscriminately without proper regard to the pH value of the soil.

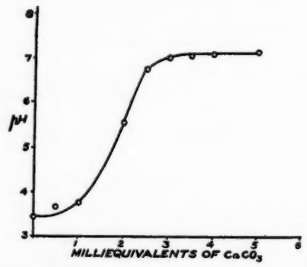

FIG. 5. TITRATION CURVE OF H-SOIL WITH CALCIUM CARBONATE

(Weight of soil = 5 gm.)

SUMMARY

Experimental evidence has been brought forth to show that soil acidoids and single-base soils behave like true acids and salts respectively in their reaction with carbonates of alkali and alkaline earths.

The merits and limitations of the method of finding lime requirement of soils based on the reaction with $CaCO_3$ are discussed. The limitations of the ratio known as "lime status" of soils are brought out.

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ORGANIC PHOSPHATES: I. FIXATION STUDIES WITH THREE DIFFERENT SOIL TYPES[1]

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Spencer and Stewart's (7, 8) investigations on soil penetration of some organic and inorganic phosphates indicate that organic phospates have promise as phosphatic fertilizers, particularly in places where deep fertilization is desirable. Their studies included percolation experiments in which a 7-inch column of soil with a top-dressing of phosphate was subjected for 3 days to water in amounts equivalent to a 24-inch rainfall.[3] Although several organic phosphates were investigated, only one type of soil, Las Vegas loam (3), occurring in an arid region where alkali is widespread, was used in the work. The organic phosphates employed were derivatives of aliphatic polyhydric alcohols as, for example, glycerol, glucose, and sorbitol, and were used in the form of the calcium salts. Analysis of the water solutions passing through the soil demonstrated that whereas 88–99 per cent of the inorganic phosphate was fixed, only 5–20 per cent of the organic compounds were retained by the soil. These results show that the major portion of the organic phosphate remained in the soluble condition for at least a short time[4] in this particular soil and therefore penetrated it to a greater extent than did the inorganic phosphates.

To examine more critically the potentialities of organic phosphates as fertilizers, it seemed desirable to study their behavior in different soil types and under a variety of soil conditions. Fixation by combination with calcium carbonate and certain iron and aluminum compounds present in soil is not possible for most phosphates of the polyhydric aliphatic compounds so long as they remain in this form, since such salts are soluble in water. A different

[1] In this article the term "phosphate fixation" is used to designate any change to a water-insoluble form. Phosphate in the "insoluble" condition may be considered to be fixed positionally; that is, penetration of the soil to a great depth would not be expected. "Fixed or insoluble phosphate" as used by us does not imply that the phosphate is unavailable for plant growth.

[2] The authors are indebted to K. D. Jacob, W. H. Ross, and L. M. White for facilitating the execution of this work and for the aid received in discussions.

[3] The extent to which organic phosphates will diffuse into the soil under more normal conditions is a quite different matter.

[4] Although the columns of soil were leached with water for 3 days, the major portion of the organic phosphate was probably in the first quarter of the leachings and was therefore in contact with the soil for less than a day. This view is supported by our results listed in table 1.

kind of fixation, involving a reaction with the colloidal matter of the soil, is more likely, but there is no means of determining *a priori* the extent to which this might take place. To obtain some information on this possibility, percolation studies were carried out with Cecil and Norfolk soils which contain widely different amounts of different kinds of colloids. Another important factor that would be expected to influence the fate of organic phosphates is the action of soil microörganisms. The organic phosphates that have so far been suggested as potential fertilizers may be assimilated by bacteria and fungi, since the organic part of these molecules can be utilized as a source of energy. If this is the case, that portion of the phosphate not required for metabolic processes will be liberated and eliminated in the inorganic form and converted to an insoluble state in a high phosphate-fixing soil. Still another path whereby the organic phosphate might be converted to inorganic phosphate is one involving catalytic hydrolysis by phosphatases, enzymes prevalent in living matter. As enzymatic hydrolysis or attack by microörganisms of organic phosphates is relatively slow, very little bacterial action could have taken place under the conditions of Spencer and Stewart's experiments.[5] Time studies were therefore included in our work in order, first, to provide the organisms with an opportunity to attack the organic phosphates and, second, to obtain information on the rate of conversion of soluble to insoluble phosphate. Comparative experiments on sterilized soil were performed with the object of obtaining evidence as to whether or not microörganisms are responsible for the gradual change of soluble to insoluble phosphate. One other factor, the variation in the pH of the soil solution, which might possibly influence the fixation of organic phosphate was also investigated. As the different organic phosphates investigated by Spencer and Stewart yielded similar results, only one, glycerophosphate, was employed in this preliminary study.

Early in the work, it was apparent that our results on Cecil and Norfolk soils were very different from those reported by the previous investigators on Las Vegas soil. In order to prove that the difference was due to the character of the soils rather than to the experimental procedures, a study of Las Vegas soil was also undertaken.

EXPERIMENTAL

Materials

Sodium glycerophosphate from the Eastman Kodak Company was found to be pure enough for our purposes. Colorimetric estimation showed only traces of inorganic phosphate to be present. The analyses were as follows: calculated for $Na_2C_3H_7PO_6 \cdot 5H_2O$—P 10.13, H_2O 29.42; found—P 10.67, H_2O 26.49. Evidently the glycerophosphate was not wholly in the pentahydrate form. The monopotassium phosphate was an analytically pure specimen.

Norfolk sandy loam and Cecil clay loam were collected in South Carolina,

[5] See footnote 3. In such a short contact period bacterial action could have been only slight.

the former in Edgefield County and the latter in Abbeville County. Norfolk soil was taken from the surface to a depth of 14 inches, and Cecil soil, from a depth of 8 to 35 inches. Las Vegas (3) loam was obtained[6] from Clark County, Nevada. According to information supplied by Spencer, the soil used in his work and in ours came from virtually the same location.[7] The soil was taken from the A horizon. Norfolk soil contained 6.3 per cent clay, 4.2 per cent colloid (included in clay), and 8.5 per cent silt; Cecil soil, 47.2 per cent clay, 41.3 per cent colloid (included in clay), and 16.2 per cent silt; and Las Vegas soil, 1.4 per cent fine gravel, 2.8 per cent coarse sand, 2.5 per cent medium sand, 40.8 per cent fine sand, 25.3 per cent very fine sand, 14.8 per cent silt, 11.6 per cent clay, and 0.4 per cent organic matter; pH of Las Vegas soil, 8.4.[8] Studies on the chemical composition of the colloid in Cecil soil and in Norfolk soil have been reported respectively by Byers, Alexander, and Holmes (2) and by Holmes, Hearn, and Byers (5). The fractions of air-dried Norfolk soil and Cecil soil passing through a 1.90-mm. sieve, and the fraction of Las Vegas soil passing through a 1.27-mm. sieve were used in this work. Specimens of the air-dried Norfolk, Las Vegas, and Cecil soils, when heated at 110° to constant weight, lost 0.1, 0.9, and 2.3 per cent of their weights respectively.

Analytical procedure

Phosphorus determinations were made in duplicate either colorimetrically or titrimetrically. Results obtained by colorimetric estimation checked with those obtained by titrimetric estimation (1, p. 19–21). In the organic phosphate experiments, the latter method included a preliminary magnesium nitrate fusion. Fiske and Subbarow's (4) micromethod for the estimation of phosphorus was used in colorimetric determinations. Before analysis, the soil extracts were digested with perchloric acid according to the directions of King (6). Not only was the organic phosphate converted to inorganic phosphate, but also the soil extracts from Las Vegas and Norfolk soils, usually yellowish brown, were decolorized, allowing accurate comparison with the standard solution. Sterilization of Las Vegas soil intensified the color of the soil extracts considerably, but no analytical difficulties were encountered. Perchloric acid digestion of Las Vegas soil extracts always resulted in the deposition of some silica. Cecil soil extracts were colorless, and digestion with perchloric acid was not necessary in the inorganic phosphate experiments.

Procedure for carrying out fixation experiments

The experimental method used in this investigation was a modification of that described by Spencer and Stewart. In order to accommodate the time

[6] Through the courtesy of C. E. Kellogg, of the Soil Survey Division.

[7] From its location on the alkali map (3) the soil would be expected to possess from 0.4–0.5 per cent alkali salts.

[8] This information was supplied by H. G. Byers, of the Soil Chemistry and Physics Research Division.

experiments, the column of soil was wet with the phosphate solution and kept in the moist condition for the required length of time before being washed thoroughly with water to remove soluble phosphate. The operation of leaching either Norfolk or Las Vegas soil was very smooth. Cecil soil, because of its high colloid content, could not be leached very effectively, and it was necessary to employ a washing and decantation process to remove the soluble phosphate.

Norfolk soil.—After preliminary trials, the following procedure was employed for most of the experimental work recorded for Norfolk soil. Four hundred grams of air-dried soil was placed in a filtering tube, $1\frac{3}{4}$ inches in diameter and 16 inches in length from the fritted glass filter plate to the top of the tube. The column of soil, about 6 or 7 inches high, was treated with 50 cc. of an aqueous solution containing 0.755 gm. sodium glycerophosphate (equivalent to 0.08 gm. phosphorus), which was the concentration used by Spencer and Stewart. Additional water was added in order to wet the column of soil completely. Both ends of the tube were then stoppered. In a few

TABLE 1

Distribution of phosphorus in soil leachings from Norfolk soil

ORDER OF WASHING	pH	P	PER CENT OF ADDED P
		mgm.	
1	6.6	44.6	55.8
2	7.2	6.1	7.6
3	7.3	2.6	3.3
4	7.3	1.6	2.0
5	7.2	1.2	1.5

experiments, the solid phosphate was added to the surface of the soil, as was done in Spencer and Stewart's work, and the column was moistened with water. Comparative experiments demonstrated that this minor variation in procedure did not affect appreciably the results. Theoretically, however, one would expect a slow reaction to be favored by the addition of phosphate in solution since it will be more evenly distributed throughout the column of soil, and under more carefully controlled long-time experiments significant differences may reveal themselves. The moist soils were allowed to stand at room temperature for various lengths of time, generally 2 to 4 hours, 3, 6, 12, or 24 days, and then each was leached with five 100-cc. portions of water. Representative results of phosphate and pH determinations obtained from the successive fractions draining from Norfolk soil in a 3-day experiment are given in table 1. Inspection of the table shows that by far the greater portion of the phosphate extracted by 500 cc. of water is present in the first 100-cc. portion. The time required to wash the soil varied from 3 to 6 hours. Usually, the longer the moist soil had stood, the slower was the filtration. The combined cloudy leachings were made up to 500 cc., centrifuged, and, if necessary, clarified by

filtration through a thin layer of celite.[9] An aliquot of this solution was subjected to analysis as soon as convenient. If the determination could not be performed immediately, the solution was kept in an ice chest until ready for use. The results obtained in the time studies using sodium glycerophosphate and Norfolk soil are shown in curve I, figure 1. The data were reproducible when the temperature at which the percolation tube stood was constant. Carrying out experiments at temperatures 5° apart, however, influenced the results significantly.

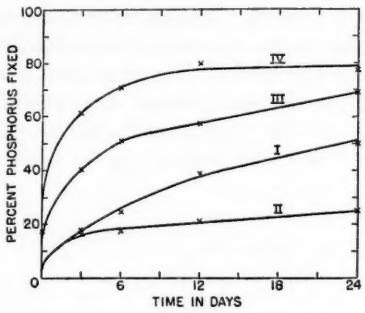

FIG. 1

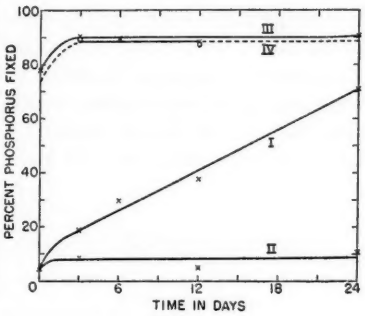

FIG. 2

FIG. 1. TIME DEPENDENCE CURVES OF IMMOBILIZATION OF ORGANIC AND INORGANIC PHOSPHATES BY RAW AND STERILIZED NORFOLK SOIL

Curve I. Sodium glycerophosphate in raw soil.
Curve II. Sodium glycerophosphate in sterilized soil.
Curve III. Monopotassium phosphate in raw soil.
Curve IV. Monopotassium phosphate in sterilized soil.

FIG. 2. TIME DEPENDENCE CURVES OF IMMOBILIZATION OF ORGANIC AND INORGANIC PHOSPHATES BY RAW AND STERILIZED LAS VEGAS SOIL

Curve I. Sodium glycerophosphate in raw soil.
Curve II. Sodium glycerophosphate in sterilized soil.
Curve III. Monopotassium phosphate in raw soil.
Curve IV. Monopotassium phosphate in sterilized soil.

The comparative study with monopotassium phosphate was carried out in a similar manner. A solution of 0.351 gm. of phosphate (equivalent to 0.08 gm. phosphorus) in water was employed to wet the soil. The leaching operations in these experiments required less time, being completed within 3 hours. The data obtained on the Norfolk soil are compiled in curve III, figure 1.

Phosphate fixation by Norfolk soil, where the pH of the soil solution was varied, was studied by employing the foregoing procedure with the following change. Before the phosphate solution containing either inorganic or organic phosphate was added to the soil, the pH of the solution was adjusted to 5, 6,

[9] Control experiments in which leachings were clarified without celite gave virtually the same results as experiments involving the use of celite.

or 7 by addition of hydrochloric acid. The pH was determined electrometrically. One series of treated soils was allowed to stand for 3 days and another for 6 days before the soluble phosphate was collected. From the results shown in table 2 it is apparent that the variation of the pH of the organic phosphate solutions added to the soil had only a minor effect on the degree of fixation in a 3- or 6-day contact period. Increasing the time of contact, however, would be expected to yield greater differences, inasmuch as the pH affects the activity of phosphatases and microörganisms.

Las Vegas soil.—With Las Vegas soil, the experimental investigation was limited to a study of the time dependence of fixation. The procedure described for Norfolk soil was used except that the sodium glycerophosphate solution was adjusted to a pH of 6.0 before addition to the soil. Because of the relatively high alkali salt content of Las Vegas soil, the combined extracts were basic, and the pH ranged from 7.7 to 7.9. The time required to wash the column of soil varied from 5–7 hours. The results obtained in the sodium

TABLE 2

Variation in fixation of KH_2PO_4 and of sodium glycerophosphate with pH in Norfolk soil

pH	KH_2PO_4		SODIUM GLYCEROPHOSPHATE	
	Per cent of P fixed		Per cent of P fixed	
	In 3 days	In 6 days	In 3 days	In 6 days
5	43.6	44.8	21.9	28.6
6	38.9	45.2	24.5	38.6
7	35.6	50.9	21.4	34.2

glycerophosphate and monopotassium phosphate experiments are shown in curves I and III, figure 2.

Sterilized Norfolk and Las Vegas soils.—Norfolk and Las Vegas soils were heated at 175°C. for 20 hours. Although some spores may have survived, there can be but little doubt that the major portion of the living matter was destroyed by this treatment. Solutions of sodium glycerophosphate and of monopotassium phosphate were prepared by addition to boiled distilled water. The glassware was sterilized by the usual heat process. Precautions were taken to prevent contamination by bacteria and molds during the various operations. The variation of fixation with time was studied according to the general method already described. Graphs of the results with sodium glycerophosphate and with monopotassium phosphate are presented in figure 1, curves II and IV, and in figure 2, curves II and IV.

Cecil soil.—Considerable difficulty was encountered in working with Cecil soil. Water or solutions having a pH of 5 to 7 percolated through a column of soil extremely slowly, whereas more basic solutions did not pass through at all. In the former case, the flow of liquid ceased after 25 to 50 cc. of filtrate had been collected. Addition of water as a fine spray to dampen the soil

and swell the colloid before placement in the tube, and application of either suction or 40 pounds pressure per square inch enhanced percolation moderately. Even under these conditions, however, the procedure was not practical, and reliance was not placed in the phosphate values obtained in the relatively small amount of filtrate. The fact that only traces of phosphate were found was later shown to be significant.

A more reliable, although laborious, procedure was developed for determining the fixation of phosphates by this type of soil. Samples of 100 gm. of the Cecil soil were placed in 250-cc. centrifuge bottles and wet with the phosphate solutions. The solution of sodium glycerophosphate consisted of 0.189 gm. of the phosphate, 20 cc. of water, and sufficient hydrochloric acid to adjust the pH to 6.0. The solution of monopotassium phosphate was prepared by dissolving 0.088 gm. of the phosphate in 20 cc. of water. After the soil was treated with the phosphate solution, additional water, usually 15 cc., was supplied to

TABLE 3

Fixation of organic and inorganic phosphate by Cecil soil

PHOSPHATE	CONDITION OF CECIL SOIL	TIME	P IN EXTRACT	PER CENT OF P FIXED
		days	*mgm.*	
Sodium glycerophosphate	Raw	3	0.93	95.3
	Raw	12	0.08	99.6
	Sterilized	3	2.16	89.2
	Sterilized	12	0.08	99.6
KH_2PO_4	Raw	3	0.40	98.0
	Raw	12	trace	99.9
	Sterilized	3	0.50	97.5
	Sterilized	12	trace	99.9

moisten the soil completely. After the soils had stood for either 3 or 12 days, 90 cc. of water was added, the mixture was thoroughly shaken for about 30 minutes, when a homogeneous mass was secured, and centrifuged. The supernatant liquid was removed and filtered. This process was repeated three more times. Phosphorus determinations were made on the combined aqueous fractions, the pH of which varied from 6.6–7.0. Typical results obtained in studies on raw and on heat-sterilized Cecil soil are shown in table 3.

RESULTS AND DISCUSSION

Our investigation demonstrates that the fixation of glycerophosphate by soils varies markedly from soil type to soil type. Only a minor amount of the organic phosphate as compared to 90 per cent of the inorganic phosphate was fixed by Las Vegas soil when the time of contact between the phosphate and the soil was 3 days or less (fig. 2). These results essentially confirm those of Spencer and Stewart on this type of soil. In the same length of time, 18 per

cent of organic phosphate and only 40 per cent of inorganic phosphate were fixed by Norfolk soil[10] (fig. 1). Under similar conditions, Cecil soil removed virtually all the organic and inorganic phosphate from the soil solution. The trend in the fixation of phosphate by the last two soils follows their colloid content, Norfolk soil containing 4.2 per cent and Cecil soil about 41.3 per cent. The data indicate that these three different soils are particularly well adapted for the study of organic phosphates, since they provide examples of the extreme as well as intermediate degrees of phosphate fixation.

The fixation of organic phosphate by Las Vegas or Norfolk soil was markedly augmented as the contact period was increased. In figure 2, fixation of organic phosphate (curve I) and fixation of inorganic phosphate (curve III) by Las Vegas soil are plotted against time. From an inspection of curve III, it is apparent that the major portion of the inorganic phosphate, in fact 78 per cent in 2 to 4 hours, was removed very rapidly, and that after a few days, no further increase in fixation took place. In contrast to the rapid fixation of inorganic phosphate only 4 per cent of the organic phosphate[11] was fixed in 2 to 4 hours. From this point on, the amount fixed increased continuously and almost linearly with time. After 24 days more than 70 per cent of the original organic phosphate had been converted to an insoluble form. The data from the time studies on Norfolk soil (fig. 1, curves I and III) show that fixation of inorganic phosphate (curve I) was more gradual and much slower than that in Las Vegas soil. In the early stages a smaller quantity of organic phosphate as compared to inorganic phosphate was fixed by Norfolk soil. It is interesting, however, that, after the sixth day, the rate of organic phosphate fixation was virtually the same as the rate of fixation of inorganic phosphate. Cecil soil fixed organic and inorganic phosphate so rapidly and completely that elaborate time studies were not necessary; 95–98 per cent of the inorganic or organic phosphate was fixed in 3 days, and only traces of phosphate remained in solution after 12 days (table 3).

Evidence, favoring the view that the increase in fixation of organic phosphate with time is in large part due to the action of microörganisms on organic phosphate with the liberation and subsequent fixation of the inorganic phosphate,

[10] It is to be pointed out that the A horizon of Norfolk sandy loam, the B horizon of Cecil clay loam, and the A horizon of Las Vegas loam were used in this work. Throughout this article, these soils have been named, for the sake of brevity, *Norfolk soil*, *Cecil soil*, and *Las Vegas soil*, respectively. When these terms are used in making comparisons we do not wish to infer we are comparing, for example, the relative fixation capacities of *Norfolk sandy loam* and *Cecil clay loam*. Instead we are interested in knowing at this stage of the investigation what the behavior of organic phosphate is in two different types of soil (Cecil soil and Norfolk soil) which, because they possess different quantities and kinds of colloidal matter, fix inorganic phosphate in different degrees.

[11] The term "organic phosphate fixation" is used rather loosely in this article, since the phosphate fixed may be in the inorganic form and have resulted from the degradation of the organic phosphate in solution.

was obtained in the studies with heat-sterilized soil. Besides destroying living matter and enzymes, heat sterilization has some effect on the physical and chemical characteristics of the inorganic soil constituents. Parallel experiments conducted with inorganic phosphate indicated the extent of the effect heat had on the fixation capacity of the soils. Curves IV, figures 1 and 2, show that the fixation capacity of Las Vegas soil was scarcely affected, whereas the fixation capacity of Norfolk soil was appreciably increased. Quite different results were obtained with organic phosphate. Fixation was much less in sterilized soil than in raw soil. This was particularly true for Las Vegas soil, where, except for a rapid initial fixation amounting to a few per cent, virtually no increase with time took place. After 24 days only 10 per cent of the phosphate had been fixed as compared to 70 per cent by the raw soil. In sterilized Norfolk soil, the amount of organic phosphate fixed increased very slowly from about 16 per cent in 3 days to about 25 per cent in 24 days. In this case, fixation at 24 days was half that occurring in raw soil for the same period of time. The rapidity with which organic phosphate was removed under normal conditions from solution by Cecil soil would seem to exclude bacterial action and suggests instead its direct combination with the colloidal matter in the soil. As was anticipated, sterilization of this soil did not change appreciably its capacity to fix either organic or inorganic phosphate. From these results as a whole, it is apparent that the action of microörganisms is an important factor in determining the fate of organic phosphates in some soils.

Although only three different types of soil have so far been investigated, it is evident from the results that the composition of the soil is an important factor in determining the extent of fixation of orthophosphates of the polyhydric aliphatic alcohols. Soil high in a certain type of colloidal matter will fix organic phosphates almost as completely as inorganic phosphate. Since this is a rapid process it is doubtful whether glycerophosphate will penetrate such soil to a greater degree than will inorganic phosphates. Soils containing much smaller amounts and a different type of colloidal matter, as Norfolk soil in which immobilization of organic phosphate increases slowly with time, will be penetrated more easily. The soluble organic phosphate, however, will be transformed to inorganic phosphate by the action of microörganisms and, therefore, will be diverted ultimately to the same fate as inorganic phosphate. Calcareous soils, such as Las Vegas soil, will rapidly convert inorganic phosphates to an insoluble condition but will not immediately affect the major portion of the organic phosphate. In this case also the phosphate will gradually be attacked by microörganisms, and as the inorganic phosphate is liberated, it will be immobilized. Although a superficial examination of our results suggests that organic phosphates would have no greater value than inorganic phosphates as phosphatic fertilizers, we do not wish to draw this conclusion at present, since other lines of investigation, still open, may yield more promising results. For example, organic orthophosphates of a different

general type from those thus far studied or certain organic metaphosphates may possibly be stable in soil over long periods of time and may not be fixed by some inorganic phosphate fixing soils.

SUMMARY

Comparative experiments were carried out on the immobilization of organic and inorganic phosphate by three different types of soil; namely, Cecil soil, Norfolk soil, and Las Vegas soil.

The fixation of glycerophosphate varied widely with the nature of the soil. In Cecil soil, which possessed about 40 per cent colloidal matter, the organic phosphate was converted almost as rapidly and completely to an insoluble form as was inorganic phosphate.

In Norfolk and Las Vegas soils, the percentage of organic phosphate immobilized increased as the time of contact of the organic phosphate with the soil increased.

Very little increase in fixation of organic phosphate by sterilized Norfolk and Las Vegas soils took place in a contact time of 3 to 24 days, thus supporting the view that the increase in fixation in raw soil is in the main a secondary reaction following the primary action of soil microörganisms on the organic phosphate.

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BOOK REVIEWS

Principles and Practice of Bacteriology. By ARTHUR H. BRYAN AND CHARLES BRYAN. Barnes & Noble, Inc., New York, 1938. Pp. 267, figs. 101. Price: cloth $2.25, paper $1.25.

The field of applied bacteriology is constantly expanding. Because of that, there is need for clear-cut guidance, not alone for the student, but also for the worker in control laboratories. The authors of the present work have had this in mind. According to their own statement:

> *Principles and Practice of Bacteriology* presents a concise, concrete, and practical reference guide and test for students and workers in both the theory and practice of bacteriology. While no originality is claimed for its content, the unique plan of organization should be of inestimable value to users of the book.
>
> Because bacteriology is basically a laboratory subject, many useful techniques, devices, and practical procedures have been included for both beginning students and practicing bacteriologists.
>
> Quick reference tables and charts, being especially helpful in bacteriology, have been added to the routine subject matter wherever important information may be needed at a glance. The tables include the pathogenic hyphomycetes and yeasts, primary determinative culture media, plant diseases, soil micro-biology, dairy bacteria, veterinary pathogens, human pathogens, dental bacteria, colon typhoid differentiation, and tables of toxins and antitoxins.

The body of the text is made up of three parts; namely, Introduction, Human Pathogenic Bacteriology, and Serology and Immunology. There are altogether 28 chapters, the titles of which are: History of Bacteriology; General Characteristics of Bacteria; Preparation and Inoculation of Culture Media; Physiological and Chemical Characters as Determinative Procedures; Methods of Destruction of Bacteria; Practical Methods of Examination of Bacteria; Yeasts, Molds, and Actinomycetes including Pathogens; Chromogens; Agricultural and Industrial Microbiology; Dairy, Air, Dust, and Water Bacteriology; Veterinary Bacteriology; Introduction to Pathogenic Bacteriology; The Pyogenic Cocci; The Specific Cocci; The Diphtheria Group; The Tuberculosis or Mycobacteria Group; The Pasteurella Group; Hemophilus Group; The Colon-Typhoid Dysentery Group; The Anaerobic Spore Bearers; Vibrio Group; The Spirochetes; Filters and Filtrable Virus Diseases; Pathogenic Protozoa; Infection and Immunity; Some Useful Serological and Diagnostic Test Methods; Toxin and Antitoxin Tables; and Animal Inoculation, Autogenous and Stock Vaccines. There are 8 appendixes and an index.

Both the teacher and the laboratory worker will welcome this book as a desirable addition to available teaching material.

Soils and Soil Management. By CHARLES ERNEST MILLAR. Webb Book Publishing Co., St. Paul, Minn., 1937. Pp. 477, figs. 248, tables 35.

There is a tendency at the present time to stress the management factor of soil study. This may be accounted for in part by local regional and national programs relating to land use. It may also be accounted for by the wide interest in soil conservation including the control of soil erosion.

The author was well aware of this fact when he was writing his book. As he himself notes:

> In preparing this text the author has attempted to analyze each soil problem as it might arise on the farm, in the garden, or about the city or country home, before beginning a discussion of ways of solving the problem. It is felt that this practice in analyzing each situation as it presents itself has an educational value beyond the specific information acquired. In addition the author has found it an interesting method of presenting subject matter to his classes.

The volume contains 26 chapters, which the author has named: Selecting a Farm; Observe Soil Formation; Classifying Soils; Fitting the Seed Bed; Maintaining Good Tilth; Cultivating the Crop; Controlling Soil Moisture; Rotating Crops; Making Commercial Fertilizer; Fitting the Fertilizer to the Soil; Fitting the Fertilizer to the Crop; Buying Fertilizers; Applying Fertilizer; Keeping the Soil from Washing; Controlling Soil Blowing; Supplying the Soil with Organic Matter; Building up Soil with Manure; Increasing Profits with Lime; Farming Acid Soils; The Alkali Problem; Adding Nitrogen to the Soil; Studying Soil Life; Managing Sandy Soils; Improving the Pasture; Gardening; and The Home Grounds. There are also a list of laboratory equipment, a glossary, and an index.

It is evident that the author has made an earnest effort to provide helpful information to the landowner. He has indicated effective methods of approach in dealing with the soil problems in different regions and has supplied the reader with references to publications that will readily supplement the information recorded in this book.

Farm Economics. By FRANK APP AND ALLEN G. WALLER. J. B. Lippincott Co., New York, 1938. Pp. 700, figs. 248.

The present volume is based on an earlier book on farm economics, by Frank App. It has the merit of dealing with farm economics, management, and distribution in a progressive way. None the less, the authors have evidently had in mind the preparation of their material in such fashion as to be particularly serviceable to producers in the northeastern region of the United States. They are well aware of the rapid changes that have taken place in the organization of both production and marketing. In their own words:

> Agriculture in America is recognized as a business. The conduct of this business is of concern not only to those directly engaged in it, but also to the rest of the nation. The invention and use of modern machinery, the building of great cities and the settlement of

our fertile lands make continual changes necessary in the economics of our agricultural production and distribution. The progress of the nation to a large measure depends upon the ability of a portion of the people to produce the food and clothing material necessary for themselves and families and to have left sufficient for several other families. This releases a portion of the population for other pursuits and in turn allows for the progress of civilization.

They also note:

In recent years a vast amount of data have been accumulated which can be used as a measure in gauging farm enterprises. The individual engaged in agriculture must understand not only the physical and biological factors necessary for sound farm enterprise, but also how to apply his farm practice so as to obtain the greatest returns from a farm enterprise. The student may need to consider the ability of the operator to handle such a business. However, the authors have not attempted to measure efficiency in terms of the human operator, but rather in terms of economies and management.

The book contains 19 chapters and an appendix. The topics dealt with are as follows: Farm Life; Size of Farm Business; Production; Farm Balance; Farm Capital and Credit; Types of Farming and Farm Enterprises; Selection of Crops; Selection of Livestock; Farm Layout and Arrangement; Acquiring a Farm; Land Rental; Farm Accounts; Cost Accounts; Cost of Producing Farm Products; Prices of Farm Products; Marketing of Farm Products; Fundamental Principles of Coöperation in Agriculture; Farm Power and Equipment; and Farm Labor.

The authors have undoubtedly rendered a service to the teacher as well as to the progressive farmer. There is a wealth of information in the book. The chapters are followed by lists of questions that should encourage thoughtful inquiry among the readers. The reference material is ample for helping the student to expand his knowledge.

Landslides and Related Phenomena. By C. F. Stewart Sharpe. Columbia University Press, New York, 1938. Pp. xii + 137, plates 9, figs. 16. Price $3.00.

This book is particularly timely in view of the nation-wide organization of soil conservation programs. The student of soils is aware of the fact that there is a type of erosion which has been designated as *geological* erosion. It involves mass movements of soil and rock and is the broader phase of the movement of surface material to lower levels. Both water and air currents are major factors in determining the character of the transportation of soil material.

The author of the present volume has tried to dramatize his subject and has done it successfully. In the words of Douglas Johnson, who wrote the foreword:

Imagine a landmass upon which no rain falls, no rivers flow, no glaciers form, no waves beat, no winds blow. Let chemical and mechanical disintegration disrupt the rocks, and gravity exert its downward pull. On such a landmass earth and rock will move ceaselessly

from higher to lower levels, slopes will soften, relief will fade. Given time enough, the whole will be reduced to a featureless plane of disintegrated rock debris.

A study of the mass-movement of rock and rock debris on such a land would be full of interest for the geomorphologist. How much more interesting is the study on such an earth as ours, where water and ice in varying quantities give endless variety to the processes and forms involved. It is to these processes and their resulting forms that Dr. Sharpe turns his attention in the pages which follow.

To the above may be added the statement of the author himself. He says:

The importance of landslides and other types of mass-movement in the denudation of the lands has long been underestimated. Some of these movements progress so slowly that their presence is scarcely realized. Others, sudden or catastrophic in nature, are overlooked because our uniformitarian concept of geology stresses the continuous action of four agencies; running water, winds, waves, and moving ice. Landslides and related phenomena are not isolated processes but are part of a continuous series grading from dry detritus movement into fluvial and glacial transportation. Any adequate classification of landslide phenomena must include related movements of flow type and must show the relation of the entire group to fluvial and glacial purposes.

The body of the book is made up of a foreword by Douglas Johnson, a preface by the author, and 7 chapters followed by a selected bibliography and an index. The titles of the several chapters are: Significance of Mass-Movement; Types of Mass-Movement; Slow Flowage; Rapid Flowage; Landslide; Subsidence; and Conclusion.

The author has dealt with his subject ably. He has developed some new points of view and has established certain conceptions which will provide a most helpful guide to students of surface geology and of land use.

Plant Biochemistry. Revised Edition. By WILLIAM EDWARD TOTTINGHAM. Burgess Publishing Co., Minneapolis, Minn., 1937. Pp. 249, figs. 98, tables 104. Price $4.25.

The biochemistry of plants has been made the subject of some of the newer research projects. Interest in biochemistry is traceable to studies of plant nutrition. Water and sand culture methods are being employed in many laboratories. In fact, the term *hydroponics* was invented to describe the growing of plants in artificial culture media. Aside from plant nutrition, there is the question of animal and human nutrition. There is also the question relating to the use of plant products in the manufacture of various industrial commodities. Here we find the term *chemurgy* used frequently. Altogether, therefore, plant biochemistry may be considered a rapidly expanding field for the investigator, the teacher, and the industrialist.

The author is well aware of the accumulation of new data and information. He says in his preface:

The rapid development of the subject has necessitated rather extensive annual revision of the notes from which this text has evolved. This indicates to be sure, that relatively frequent revision of text books in the field will be necessary for some time. Fortunately, this task

stands likely to be much lightened by the annual reviews of biochemistry which have developed. Citations of the literature have been introduced freely, partly as evidence to support positions taken by the author and partly to induce breadth of reading on the part of the student. Much care has been exercised in consulting the original articles for accuracy as to both citation and reference. An opportunity to revise the original edition has been used for amplifying and modernizing discussional features.

The book contains 10 chapters and an appendix. For the convenience of the reader, the titles used to designate the several chapters are herewith given. They are: General Aspects; The Photosynthesis; the Metabolism of Carbohydrates; Fat Metabolism; The Metabolism of Nitrogen Compounds; Physico-Chemical Relations of the Plant Cell; the Nature and Function of Enzymes; Chemical Aspects of Respiration; Salt Nutrition; and Climatic Effects in Metabolism. The materials of metabolism are discussed in the appendix.

It will be noted that considerable attention has been given to the general subject of metabolism, including that related to carbohydrates, fats, and nitrogen compounds. The nature and functions of the plant cells, the nature and functions of enzymes and of mineral salts, and, likewise, the influence of climatic environment on metabolism have been reviewed by the author. The more important references are also given.

From the instructor's point of view, *Plant Biochemistry* should prove decidedly helpful.

The Chemistry of Plant Constituents. By OLE GISVOLD AND CHARLES H. ROGERS. Burgess Publishing Co., Minneapolis, Minn., 1938. Pp. 309, tables 28. Price $3.50.

The number of compounds found in plants is without end. This will account for the specialized approach that is adopted by many compilers of data on the chemistry of plant constituents. Gisvold and Rogers' conception of their task is indicated as follows:

The subject matter of this book treats of the chemistry of plant constituents and some of the theories advanced to explain their formations. Various probable and also possible reactions that may be involved in the formation of the many known and characteristic parts of large molecules found in plants have been presented and discussed. The very fact that, starting with first or simple materials, a plant is able to synthesize complex molecules through the agencies necessary to its development would indicate that a myriad of complicated reactions take place. To postulate a theory for the formation of an alkaloid of complex composition from one of the first products of photosynthesis, formaldehyde, might appear at first to be beyond the scope of the most vivid imagination. Nevertheless, many syntheses like this one are actually accomplished by plants and, perhaps, by the presentation of many theories, a few may offer to science explanations of transitions which at times have seemed to be enigmatical. Also, attention is directed to certain organic reactions that may either be involved in the formation of plant constituents or aid in characterizing and proving the structures of these products of plant life.

The treatise contains 15 chapters and a bibliography. The chapters are named, respectively: Introduction; Carbohydrates; Vegetable Fats; Plant

and Insect Waxes; Phytosterols; Proteins; Alkaloids; Glycosides; The Anthocyanins and Anthoxanthins; Carotinoids; Tannins; Terpenes and Oxygenated Terpenes; Vitamins; Enzymes; and Products of Fermentation.

It will be noted that major consideration is given to carbohydrates, fats, waxes, phytosterols, plant substances containing nitrogen, and a number of products qualitatively important even though quantitatively they might represent a minor portion of the plant tissues. The authors summarize some of the present-day knowledge of fermentation products including alcohols and organic acids.

The student, the teacher, and the investigator will find this work a convenient source of ably compiled and interpreted data.

The Foundations of Nutrition. Third Edition. By MARY SWARTZ ROSE. The Macmillan Company, New York, 1938. Pp. xi + 625, frontispiece, figs. 116, tables 9. Price $3.50.

This is a revision of the first and second editions, published in 1927 and 1933, respectively. The extent of the revision that was necessary is described by the author. She says:

> In preparing this third edition it has been gratifying to note that in the main the researches of the past five years have only served to establish more firmly the foundations of nutrition as originally outlined in 1927. At the same time, it has been necessary to make a thorough revision of every topic because there have been many additions to our knowledge of every dietary essential. These new advances enable us to understand human requirements better and to use our common food materials to increasingly good advantage. In 1933 only three of the vitamins had been crystallized and in case of only two had any clear picture of chemical structure begun to emerge. In this edition it is possible to state not only that six have been crystallized and chemically identified, but also that three of them have been prepared artificially. The use of highly concentrated preparations or of pure crystals has helped to advance rapidly our understanding of the functions of the vitamins and great activity in the assay of foods for vitamin values has furnished a mass of data, necessitating complete revision of all tables and dietaries.

The present volume contains 26 chapters, 9 appendixes, and an index. The titles of the several chapters, which follow, will acquaint the reader with the author's approach to the subject. They are: Historical Introduction; The Body's Need for Energy and Food as the Source of Supply; The Energy Requirement of Adults; Factors Causing Variation in the Basal Metabolism of Individuals; The Energy Requirement of Children; Shortage and Surplus of Calories; Protein as Body Building Material; Mineral Elements and Water as Body Building Material; Mineral Elements and Water as Regulators of Body Processes; Vitamin A as a Regulator of Body Processes; Vitamin B (B_1) as a Regulator of Body Processes; Vitamin C as a Regulator of Body Processes; Vitamin D as a Regulator of Body Processes; Vitamin G as a Regulator of Body Processes; Vitamins of Undetermined Significance in Human Nutrition; Contributions to the Diet Made by Various Types of Food Materials; Milk; The Foods Derived from Grains; Vegetables and Fruits; Eggs,

Cheese, Nuts, Meat and Other Flesh Foods; Fat, Sugars and Other Sweets; The Construction of an Adequate Diet: Diets for Adults; Food Needs of Pre-School and Kindergarten Children; Food Needs of Elementary and High School Boys and Girls; Well Balanced Diets for Family Groups; and Special Food Needs of Mothers and Babies.

The Foundations of Nutrition may well be accepted as a valuable contribution to the field of preventive medicine. The proper nutrition of animals and of humans plays an important rôle in maintaining good health. The author has rendered a service to teachers in schools and colleges, and, beyond that, to the large group of extension workers and lecturers who are expected to keep the public acquainted with rational procedures in the purchase, the handling, and the cooking of foods as well as in the preparation of suitable dietaries.

Le Sistemazioni Idraulico-Agrarie. By ALBERTO OLIVA. S. A. G. Barbera, Firenze, Italy, 1938. Pp. 356, figs. 102.

This book is devoted to the consideration of soil erosion as it applies particularly to the soils of Italy. Many centuries of soil exploitation, soil conservation, and soil building have left a deep impression on soil management practices in that country. The traveler in Italy is early impressed by the painstaking, and often exhaustive, labor devoted to the protecting of the land against gully and sheet erosion. He will be impressed no less by the land drainage practices, by terracing, forestation, and grassland farming as a part of the soil conservation and soil erosion control movement. From that point of view, European countries have traveled much farther along the road of soil saving than have the newer countries of the two Americas. Despite all effort to the contrary, there is still a serious soil erosion problem in Italy. This is indicated by the following statement of the author:

> Soil erosion has assumed serious proportions in Italy owing to the prevalent hilly and mountainous character of the territory, to the extension of Miocene and Pliocene clays and Eocene sand, also to the violent rainfall with frequent cloud-bursts.
>
> Since very early times the requirements of Italian agriculture have necessitated the taking of measures against erosion. This explains the evolution in Italy of a special technique by means of which the more serious effects of the erosion have been successfully checked or at least mitigated.
>
> During the last half century the technique of the measures for prevention of soil erosion made further progress in the direction of conservation and ultilisation of some part of the rain water for the benefit of the crops during the periods of drought, that is to say, by establishing what the author calls "water economy." Surprising results have been achieved by this technique, especially on the Tuscan hillsides between Florence, Pisa and Siena.
>
> The protection of the soil against rain-water and the utilization for the benefit of crops is called hydraulic and agricultural regulation or simple "regulation."

We may also note from the author's statement:

> Many hitherto unknown aspects of Italian agricultural progress, and the secrets of some of the most interesting Italian landscapes are revealed in this volume. In fact the Italian soil regulation works, more than the natural forms of the land, create the orderly and artistic tone of the Italian country.

The body of the book consists of an introduction, 7 chapters, and summaries in English and German. There are also a bibliography, nomeclature, and 3 indexes. The several chapters are entitled, respectively: Concetto e limiti delle sistemazioni; De correlazioni generali delle sistemazioni e di loro particolari aspetti nei terreni di pianura; Le sistemazioni di pianura; Le sistemazioni collinari intensive o bonifiche collinari; Le sistemazioni estensive collinari e di bassa e media montagna; Direttive sulle sistemazioni del suolo declive e forme anomale; and Elementi dei costi delle sistemazioni.

Students of soils and economics will find much of interest in this book. There are also more or less direct relations to the sociological aspect of rural life.

Soil Science Society of America. Vol. II. Proceedings 1937. The Soil Science Society of America, F. B. Smith, Sec.-Treas., Gainesville, Fla., 1938. Pp. ix +602. Price $5.00.

The Soil Science Society of America is the American Section of the International Society of Soil Science. It is also an affiliate of the American Society of Agronomy.

The present volume is more than a mere collection of the papers presented at the 1937 annual meeting of the society. The Proceedings record the officers of the society, the section officers, and the names of the sections themselves. They are Soil Physics, Soil Chemistry, Soil Microbiology, Soil Fertility, Soil Morphology, and Soil Technology. Under the headings of these sections are reported a total of 102 papers. There is also a record of the business meetings, committee reports, and announcements.

Despite the limited funds for publication, the officers of the society have done very creditable work in publishing the material presented at the 1937 annual meeting. These Proceedings are, unquestionably, a distinct addition in the field of soil science, particularly as it is being developed by American workers.

JACOB G. LIPMAN